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## PROTEASES

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of proteases and to the use of these sequences in the diagnosis, treatment, and prevention of gastrointestinal, cardiovascular, autoimmune/inflammatory, cell proliferative, developmental, epithelial, neurological, and reproductive disorders, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of proteases.

### BACKGROUND OF THE INVENTION

Proteases cleave proteins and peptides at the peptide bond that forms the backbone of the protein or peptide chain. Proteolysis is one of the most important and frequent enzymatic reactions that occurs both within and outside of cells. Proteolysis is responsible for the activation and maturation of nascent polypeptides, the degradation of misfolded and damaged proteins, and the controlled turnover of peptides within the cell. Proteases participate in digestion, endocrine function, and tissue remodeling during embryonic development, wound healing, and normal growth. Proteases can play a role in regulatory processes by affecting the half life of regulatory proteins. Proteases are involved in the etiology or progression of disease states such as inflammation, angiogenesis, tumor dispersion and metastasis, cardiovascular disease, neurological disease, and bacterial, parasitic, and viral infections.

Proteases can be categorized on the basis of where they cleave their substrates. Exopeptidases, which include aminopeptidases, dipeptidyl peptidases, tripeptidases, carboxypeptidases, peptidyl-dipeptidases, dipeptidases, and omega peptidases, cleave residues at the termini of their substrates. Endopeptidases, including serine proteases, cysteine proteases, and metalloproteases, cleave at residues within the peptide. Four principal categories of mammalian proteases have been identified based on active site structure, mechanism of action, and overall three-dimensional structure. (See Beynon, R.J. and J.S. Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York NY, pp. 1-5.)

#### Serine Proteases

The serine proteases (SPs) are a large, widespread family of proteolytic enzymes that include the digestive enzymes trypsin and chymotrypsin, components of the complement and blood-clotting cascades, and enzymes that control the degradation and turnover of macromolecules within the cell and in the extracellular matrix. Most of the more than 20 subfamilies can be grouped into six clans, each with a common ancestor. These six clans are hypothesized to have descended from at least four evolutionarily distinct ancestors. SPs are named for the presence of a serine residue found in the active

catalytic site of most families. The active site is defined by the catalytic triad, a set of conserved asparagine, histidine, and serine residues critical for catalysis. These residues form a charge relay network that facilitates substrate binding. Other residues outside the active site form an oxyanion hole that stabilizes the tetrahedral transition intermediate formed during catalysis. SPs have a wide range of substrates and can be subdivided into subfamilies on the basis of their substrate specificity. The main subfamilies are named for the residue(s) after which they cleave: trypases (after arginine or lysine), aspases (after aspartate), chymases (after phenylalanine or leucine), metases (methionine), and serases (after serine) (Rawlings, N.D. and A.J. Barrett (1994) *Meth. Enzymol.* 244:19-61).

Most mammalian serine proteases are synthesized as zymogens, inactive precursors that are activated by proteolysis. For example, trypsinogen is converted to its active form, trypsin, by enteropeptidase. Enteropeptidase is an intestinal protease that removes an N-terminal fragment from trypsinogen. The remaining active fragment is trypsin, which in turn activates the precursors of the other pancreatic enzymes. Likewise, proteolysis of prothrombin, the precursor of thrombin, generates three separate polypeptide fragments. The N-terminal fragment is released while the other two fragments, which comprise active thrombin, remain associated through disulfide bonds.

The two largest SP subfamilies are the chymotrypsin (S1) and subtilisin (S8) families. Some members of the chymotrypsin family contain two structural domains unique to this family. Kringle domains are triple-looped, disulfide cross-linked domains found in varying copy number. Kringles are thought to play a role in binding mediators such as membranes, other proteins or phospholipids, and in the regulation of proteolytic activity (PROSITE PDOC00020). Apple domains are 90 amino-acid repeated domains, each containing six conserved cysteines. Three disulfide bonds link the first and sixth, second and fifth, and third and fourth cysteines (PROSITE PDOC00376). Apple domains are involved in protein-protein interactions. S1 family members include trypsin, chymotrypsin, coagulation factors IX-XII, complement factors B, C, and D, granzymes, kallikrein, and tissue- and urokinase-plasminogen activators. The subtilisin family has members found in the eubacteria, archaeobacteria, eukaryotes, and viruses. Subtilisins include the proprotein-processing endopeptidases kexin and furin and the pituitary prohormone convertases PC1, PC2, PC3, PC6, and PACE4 (Rawlings and Barrett, *supra*).

SPs have functions in many normal processes and some have been implicated in the etiology or treatment of disease. Enterokinase, the initiator of intestinal digestion, is found in the intestinal brush border, where it cleaves the acidic propeptide from trypsinogen to yield active trypsin (Kitamoto, Y. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:7588-7592). Prolylcarboxypeptidase, a lysosomal serine peptidase that cleaves peptides such as angiotensin II and III and [des-Arg9] bradykinin, shares sequence homology with members of both the serine carboxypeptidase and prolylendopeptidase families



(Tan, F. et al. (1993) *J. Biol. Chem.* 268:16631-16638). The protease neuropsin may influence synapse formation and neuronal connectivity in the hippocampus in response to neural signaling (Chen, Z.-L. et al. (1995) *J Neurosci* 15:5088-5097). Tissue plasminogen activator is useful for acute management of stroke (Zivin, J.A. (1999) *Neurology* 53:14-19) and myocardial infarction (Ross, A.M. (1999) *Clin. Cardiol.* 22:165-171). Some receptors (PAR, for proteinase-activated receptor), highly expressed throughout the digestive tract, are activated by proteolytic cleavage of an extracellular domain. The major agonists for PARs, thrombin, trypsin, and mast cell tryptase, are released in allergy and inflammatory conditions. Control of PAR activation by proteases has been suggested as a promising therapeutic target (Vergnolle, N. (2000) *Aliment. Pharmacol. Ther.* 14:257-266; Rice, K.D. et al. (1998) *Curr. Pharm. Des.* 4:381-396). Prostate-specific antigen (PSA) is a kallikrein-like serine protease synthesized and secreted exclusively by epithelial cells in the prostate gland. Serum PSA is elevated in prostate cancer and is the most sensitive physiological marker for monitoring cancer progression and response to therapy. PSA can also identify the prostate as the origin of a metastatic tumor (Brawer, M.K. and P.H. Lange (1989) *Urology* 33:11-16).

The signal peptidase is a specialized class of SP found in all prokaryotic and eukaryotic cell types that serves in the processing of signal peptides from certain proteins. Signal peptides are amino-terminal domains of a protein which direct the protein from its ribosomal assembly site to a particular cellular or extracellular location. Once the protein has been exported, removal of the signal sequence by a signal peptidase and posttranslational processing, e.g., glycosylation or phosphorylation, activate the protein. Signal peptidases exist as multi-subunit complexes in both yeast and mammals. The canine signal peptidase complex is composed of five subunits, all associated with the microsomal membrane and containing hydrophobic regions that span the membrane one or more times (Shelness, G.S. and G. Blobel (1990) *J. Biol. Chem.* 265:9512-9519). Some of these subunits serve to fix the complex in its proper position on the membrane while others contain the actual catalytic activity.

Another family of proteases which have a serine in their active site are dependent on the hydrolysis of ATP for their activity. These proteases contain proteolytic core domains and regulatory ATPase domains which can be identified by the presence of the P-loop, an ATP/GTP-binding motif (PROSITE PDOC00803). Members of this family include the eukaryotic mitochondrial matrix proteases, Clp protease and the proteasome. Clp protease was originally found in plant chloroplasts but is believed to be widespread in both prokaryotic and eukaryotic cells. The gene for early-onset torsion dystonia encodes a protein related to Clp protease (Ozelius, L.J. et al. (1998) *Adv. Neurol.* 78:93-105).

The proteasome is an intracellular protease complex found in some bacteria and in all eukaryotic cells, and plays an important role in cellular physiology. Proteasomes are associated with the ubiquitin conjugation system (UCS), a major pathway for the degradation of cellular proteins of all

types, including proteins that function to activate or repress cellular processes such as transcription and cell cycle progression (Ciechanover, A. (1994) *Cell* 79:13-21). In the UCS pathway, proteins targeted for degradation are conjugated to ubiquitin, a small heat stable protein. The ubiquitinated protein is then recognized and degraded by the proteasome. The resultant ubiquitin-peptide complex is

5 hydrolyzed by a ubiquitin carboxyl terminal hydrolase, and free ubiquitin is released for reutilization by the UCS. Ubiquitin-proteasome systems are implicated in the degradation of mitotic cyclic kinases, oncoproteins, tumor suppressor genes (p53), cell surface receptors associated with signal transduction, transcriptional regulators, and mutated or damaged proteins (Ciechanover, supra). This pathway has been implicated in a number of diseases, including cystic fibrosis, Angelman's syndrome, and Liddle

10 syndrome (reviewed in Schwartz, A.L. and A. Ciechanover (1999) *Annu. Rev. Med.* 50:57-74). A murine proto-oncogene, Unp, encodes a nuclear ubiquitin protease whose overexpression leads to oncogenic transformation of NIH3T3 cells. The human homologue of this gene is consistently elevated in small cell tumors and adenocarcinomas of the lung (Gray, D.A. (1995) *Oncogene* 10:2179-2183). Ubiquitin carboxyl terminal hydrolase is involved in the differentiation of a lymphoblastic leukemia cell

15 line to a non-dividing mature state (Maki, A. et al. (1996) *Differentiation* 60:59-66). In neurons, ubiquitin carboxyl terminal hydrolase (PGP 9.5) expression is strong in the abnormal structures that occur in human neurodegenerative diseases (Lowe, J. et al. (1990) *J. Pathol.* 161:153-160). The proteasome is a large (~2000 kDa) multisubunit complex composed of a central catalytic core containing a variety of proteases arranged in four seven-membered rings with the active sites facing

20 inwards into the central cavity, and terminal ATPase subunits covering the outer port of the cavity and regulating substrate entry (for review, see Schmidt, M. et al. (1999) *Curr. Opin. Chem. Biol.* 3:584-591).

### Cysteine Proteases

Cysteine proteases (CPs) are involved in diverse cellular processes ranging from the processing

25 of precursor proteins to intracellular degradation. Nearly half of the CPs known are present only in viruses. CPs have a cysteine as the major catalytic residue at the active site where catalysis proceeds via a thioester intermediate and is facilitated by nearby histidine and asparagine residues. A glutamine residue is also important, as it helps to form an oxyanion hole. Two important CP families include the papain-like enzymes (C1) and the calpains (C2). Papain-like family members are generally lysosomal

30 or secreted and therefore are synthesized with signal peptides as well as propeptides. Most members bear a conserved motif in the propeptide that may have structural significance (Karrer, K.M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:3063-3067). Three-dimensional structures of papain family members show a bilobed molecule with the catalytic site located between the two lobes. Papains include cathepsins B, C, H, L, and S, certain plant allergens and dipeptidyl peptidase (for a review, see

Rawlings, N.D. and A.J. Barrett (1994) *Meth. Enzymol.* 244:461-486).

Some CPs are expressed ubiquitously, while others are produced only by cells of the immune system. Of particular note, CPs are produced by monocytes, macrophages and other cells which migrate to sites of inflammation and secrete molecules involved in tissue repair. Overabundance of these repair molecules plays a role in certain disorders. In autoimmune diseases such as rheumatoid arthritis, secretion of the cysteine peptidase cathepsin C degrades collagen, laminin, elastin and other structural proteins found in the extracellular matrix of bones. Bone weakened by such degradation is also more susceptible to tumor invasion and metastasis. Cathepsin L expression may also contribute to the influx of mononuclear cells which exacerbates the destruction of the rheumatoid synovium (Keyszer, G.M. (1995) *Arthritis Rheum.* 38:976-984).

Calpains are calcium-dependent cytosolic endopeptidases which contain both an N-terminal catalytic domain and a C-terminal calcium-binding domain. Calpain is expressed as a proenzyme heterodimer consisting of a catalytic subunit unique to each isoform and a regulatory subunit common to different isoforms. Each subunit bears a calcium-binding EF-hand domain. The regulatory subunit also contains a hydrophobic glycine-rich domain that allows the enzyme to associate with cell membranes. Calpains are activated by increased intracellular calcium concentration, which induces a change in conformation and limited autolysis. The resultant active molecule requires a lower calcium concentration for its activity (Chan, S.L. and M.P. Mattson (1999) *J. Neurosci. Res.* 58:167-190). Calpain expression is predominantly neuronal, although it is present in other tissues. Several chronic neurodegenerative disorders, including ALS, Parkinson's disease and Alzheimer's disease are associated with increased calpain expression (Chan and Mattson, *supra*). Calpain-mediated breakdown of the cytoskeleton has been proposed to contribute to brain damage resulting from head injury (McCracken, E. et al. (1999) *J. Neurotrauma* 16:749-761). Calpain-3 is predominantly expressed in skeletal muscle, and is responsible for limb-girdle muscular dystrophy type 2A (Minami, N. et al. (1999) *J. Neurol. Sci.* 171:31-37).

Another family of thiol proteases is the caspases, which are involved in the initiation and execution phases of apoptosis. A pro-apoptotic signal can activate initiator caspases that trigger a proteolytic caspase cascade, leading to the hydrolysis of target proteins and the classic apoptotic death of the cell. Two active site residues, a cysteine and a histidine, have been implicated in the catalytic mechanism. Caspases are among the most specific endopeptidases, cleaving after aspartate residues. Caspases are synthesized as inactive zymogens consisting of one large (p20) and one small (p10) subunit separated by a small spacer region, and a variable N-terminal prodomain. This prodomain interacts with cofactors that can positively or negatively affect apoptosis. An activating signal causes autoproteolytic cleavage of a specific aspartate residue (D297 in the caspase-1 numbering convention)

and removal of the spacer and prodomain, leaving a p10/p20 heterodimer. Two of these heterodimers interact via their small subunits to form the catalytically active tetramer. The long prodomains of some caspase family members have been shown to promote dimerization and auto-processing of procaspases. Some caspases contain a "death effector domain" in their prodomain by which they can be recruited into  
5 self-activating complexes with other caspases and FADD protein associated death receptors or the TNF receptor complex. In addition, two dimers from different caspase family members can associate, changing the substrate specificity of the resultant tetramer. Endogenous caspase inhibitors (inhibitor of apoptosis proteins, or IAPs) also exist. All these interactions have clear effects on the control of apoptosis (reviewed in Chan and Mattson, supra; Salveson, G.S. and V.M. Dixit (1999) Proc. Natl.  
10 Acad. Sci. USA 96:10964-10967).

Caspases have been implicated in a number of diseases. Mice lacking some caspases have severe nervous system defects due to failed apoptosis in the neuroepithelium and suffer early lethality. Others show severe defects in the inflammatory response, as caspases are responsible for processing IL-1b and possibly other inflammatory cytokines (Chan and Mattson, supra). Cowpox virus and  
15 baculoviruses target caspases to avoid the death of their host cell and promote successful infection. In addition, increases in inappropriate apoptosis have been reported in AIDS, neurodegenerative diseases and ischemic injury, while a decrease in cell death is associated with cancer (Salveson and Dixit, supra; Thompson, C.B. (1995) Science 267:1456-1462).

#### Aspartyl proteases

20 Aspartyl proteases (APs) include the lysosomal proteases cathepsins D and E, as well as chymosin, renin, and the gastric pepsins. Most retroviruses encode an AP, usually as part of the pol polyprotein. APs, also called acid proteases, are monomeric enzymes consisting of two domains, each domain containing one half of the active site with its own catalytic aspartic acid residue. APs are most active in the range of pH 2–3, at which one of the aspartate residues is ionized and the other neutral.  
25 The pepsin family of APs contains many secreted enzymes, and all are likely to be synthesized with signal peptides and propeptides. Most family members have three disulfide loops, the first ~5 residue loop following the first aspartate, the second 5-6 residue loop preceding the second aspartate, and the third and largest loop occurring toward the C terminus. Retropepsins, on the other hand, are analogous to a single domain of pepsin, and become active as homodimers with each retropepsin monomer  
30 contributing one half of the active site. Retropepsins are required for processing the viral polyproteins.

APs have roles in various tissues, and some have been associated with disease. Renin mediates the first step in processing the hormone angiotensin, which is responsible for regulating electrolyte balance and blood pressure (reviewed in Crews, D.E. and S.R. Williams (1999) Hum. Biol. 71:475-503). Abnormal regulation and expression of cathepsins are evident in various inflammatory disease

states. Expression of cathepsin D is elevated in synovial tissues from patients with rheumatoid arthritis and osteoarthritis. The increased expression and differential regulation of the cathepsins are linked to the metastatic potential of a variety of cancers (Chambers, A.F. et al. (1993) Crit. Rev. Oncol. 4:95-114).

## 5 Metalloproteases

Most zinc-dependent metalloproteases share a common sequence in the zinc-binding domain. The active site is made up of two histidines which act as zinc ligands and a catalytic glutamic acid C-terminal to the first histidine. Proteins containing this signature sequence are known as the metzincins and include aminopeptidase N, angiotensin-converting enzyme, neurolysin, the matrix metalloproteases and the adamalysins (ADAMS). An alternate sequence is found in the zinc carboxypeptidases, in which  
10 all three conserved residues – two histidines and a glutamic acid – are involved in zinc binding.

A number of the neutral metalloendopeptidases, including angiotensin converting enzyme and the aminopeptidases, are involved in the metabolism of peptide hormones. High aminopeptidase B activity, for example, is found in the adrenal glands and neurohypophyses of hypertensive rats (Prieto,  
15 I. et al. (1998) Horm. Metab. Res. 30:246-248). Oligopeptidase M/neurolysin can hydrolyze bradykinin as well as neurotensin (Serizawa, A. et al. (1995) J. Biol. Chem 270:2092-2098). Neurotensin is a vasoactive peptide that can act as a neurotransmitter in the brain, where it has been implicated in limiting food intake (Tritos, N.A. et al. (1999) Neuropeptides 33:339-349).

The matrix metalloproteases (MMPs) are a family of at least 23 enzymes that can degrade  
20 components of the extracellular matrix (ECM). They are  $Zn^{+2}$  endopeptidases with an N-terminal catalytic domain. Nearly all members of the family have a hinge peptide and C-terminal domain which can bind to substrate molecules in the ECM or to inhibitors produced by the tissue (TIMPs, for tissue inhibitor of metalloprotease; Campbell, I.L. et al. (1999) Trends Neurosci. 22:285). The presence of fibronectin-like repeats, transmembrane domains, or C-terminal hemopexinase-like domains can be used  
25 to separate MMPs into collagenase, gelatinase, stromelysin and membrane-type MMP subfamilies. In the inactive form, the  $Zn^{+2}$  ion in the active site interacts with a cysteine in the pro-sequence. Activating factors disrupt the  $Zn^{+2}$ -cysteine interaction, or "cysteine switch," exposing the active site. This partially activates the enzyme, which then cleaves off its propeptide and becomes fully active. MMPs are often activated by the serine proteases plasmin and furin. MMPs are often regulated by  
30 stoichiometric, noncovalent interactions with inhibitors; the balance of protease to inhibitor, then, is very important in tissue homeostasis (reviewed in Yong, V.W. et al. (1998) Trends Neurosci. 21:75).

MMPs are implicated in a number of diseases including osteoarthritis (Mitchell, P. et al. (1996) J. Clin. Invest. 97:761), atherosclerotic plaque rupture (Sukhova, G.K. et al. (1999) Circulation 99:2503), aortic aneurysm (Schneiderman, J. et al. (1998) Am. J. Path. 152:703), non-healing wounds

(Saarialho-Kere, U.K. et al. (1994) *J. Clin. Invest.* 94:79), bone resorption (Blavier, L. and J.M. Delaisse (1995) *J. Cell Sci.* 108:3649), age-related macular degeneration (Steen, B. et al. (1998) *Invest. Ophthalmol. Vis. Sci.* 39:2194), emphysema (Finlay, G.A. et al. (1997) *Thorax* 52:502), myocardial infarction (Rohde, L.E. et al. (1999) *Circulation* 99:3063) and dilated cardiomyopathy (Thomas, C.V. et al. (1998) *Circulation* 97:1708). MMP inhibitors prevent metastasis of mammary carcinoma and experimental tumors in rat, and Lewis lung carcinoma, hemangioma, and human ovarian carcinoma xenografts in mice (Eccles, S.A. et al. (1996) *Cancer Res.* 56:2815; Anderson et al. (1996) *Cancer Res.* 56:715-718; Volpert, O.V. et al. (1996) *J. Clin. Invest.* 98:671; Taraboletti, G. et al. (1995) *J. NCI* 87:293; Davies, B. et al. (1993) *Cancer Res.* 53:2087). MMPs may be active in Alzheimer's disease.

10 A number of MMPs are implicated in multiple sclerosis, and administration of MMP inhibitors can relieve some of its symptoms (reviewed in Yong, supra).

Another family of metalloproteases is the ADAMs, for A Disintegrin and Metalloprotease Domain, which they share with their close relatives the adamalysins, snake venom metalloproteases (SVMPs). ADAMs combine features of both cell surface adhesion molecules and proteases, containing

15 a prodomain, a protease domain, a disintegrin domain, a cysteine rich domain, an epidermal growth factor repeat, a transmembrane domain, and a cytoplasmic tail. The first three domains listed above are also found in the SVMPs. The ADAMs possess four potential functions: proteolysis, adhesion, signaling and fusion. The ADAMs share the metzincin zinc binding sequence and are inhibited by some MMP antagonists such as TIMP-1.

20 ADAMs are implicated in such processes as sperm-egg binding and fusion, myoblast fusion, and protein-ectodomain processing or shedding of cytokines, cytokine receptors, adhesion proteins and other extracellular protein domains (Schlöndorff, J. and C.P. Blobel (1999) *J. Cell. Sci.* 112:3603-3617). The Kuzbanian protein cleaves a substrate in the NOTCH pathway (possibly NOTCH itself), activating the program for lateral inhibition in Drosophila neural development. Two ADAMs, TACE

25 (ADAM 17) and ADAM 10, are proposed to have analogous roles in the processing of amyloid precursor protein in the brain (Schlöndorff and Blobel, supra). TACE has also been identified as the TNF activating enzyme (Black, R.A. et al. (1997) *Nature* 385:729). TNF is a pleiotropic cytokine that is important in mobilizing host defenses in response to infection or trauma, but can cause severe damage in excess and is often overproduced in autoimmune disease. TACE cleaves membrane-bound

30 pro-TNF to release a soluble form. Other ADAMs may be involved in a similar type of processing of other membrane-bound molecules.

The ADAMTS sub-family has all of the features of ADAM family metalloproteases and contain an additional thrombospondin domain (TS). The prototypic ADAMTS was identified in mouse, found to be expressed in heart and kidney and upregulated by proinflammatory stimuli (Kuno, K. et al.

(1997) J. Biol. Chem. 272:556). To date eleven members are recognized by the Human Genome Organization (HUGO; <http://www.gene.ucl.ac.uk/users/hester/adamts.html#Approved>). Members of this family have the ability to degrade aggrecan, a high molecular weight proteoglycan which provides cartilage with important mechanical properties including compressibility, and which is lost during the development of arthritis. Enzymes which degrade aggrecan are thus considered attractive targets to prevent and slow the degradation of articular cartilage (See, e.g., Tortorella, M.D. (1999) Science 284:1664; Abbaszade, I. (1999) J. Biol. Chem. 274:23443). Other members are reported to have antiangiogenic potential (Kuno et al., *supra*) and/or procollagen processing (Colige, A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2374).

#### 10 Protease inhibitors

Protease inhibitors and other regulators of protease activity control the activity and effects of proteases. Protease inhibitors have been shown to control pathogenesis in animal models of proteolytic disorders (Murphy, G. (1991) Agents Actions Suppl. 35:69-76). Low levels of the cystatins, low molecular weight inhibitors of the cysteine proteases, correlate with malignant progression of tumors (Calkins, C. et al. (1995) Biol. Biochem. Hoppe Seyler 376:71-80). Serpins are inhibitors of mammalian plasma serine proteases. Many serpins serve to regulate the blood clotting cascade and/or the complement cascade in mammals. Sp32 is a positive regulator of the mammalian acrosomal protease, acrosin, that binds the proenzyme, proacrosin, and thereby aides in packaging the enzyme into the acrosomal matrix (Baba, T. et al. (1994) J. Biol. Chem. 269:10133-10140). The Kunitz family of serine protease inhibitors are characterized by one or more "Kunitz domains" containing a series of cysteine residues that are regularly spaced over approximately 50 amino acid residues and form three intrachain disulfide bonds. Members of this family include aprotinin, tissue factor pathway inhibitor (TFPI-1 and TFPI-2), inter- $\alpha$ -trypsin inhibitor, and bikunin. (Marlor, C.W. et al. (1997) J. Biol. Chem. 272:12202-12208.) Members of this family are potent inhibitors (in the nanomolar range) against serine proteases such as kallikrein and plasmin. Aprotinin has clinical utility in reduction of perioperative blood loss.

The discovery of new proteases and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of gastrointestinal, cardiovascular, autoimmune/inflammatory, cell proliferative, developmental, epithelial, neurological, and reproductive disorders, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of proteases.

## SUMMARY OF THE INVENTION

The invention features purified polypeptides, proteases, referred to collectively as "PRTS" and individually as "PRTS-1," "PRTS-2," "PRTS-3," "PRTS-4," "PRTS-5," "PRTS-6," "PRTS-7," "PRTS-8," "PRTS-9," "PRTS-10," "PRTS-11," "PRTS-12," "PRTS-13," and "PRTS-14." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-14. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:15-28.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c)



a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.

The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said

target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and a pharmaceutically acceptable excipient. In one embodiment, the composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional PRTS, comprising administering to a patient in need of such treatment the composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional PRTS, comprising administering to a patient in need of such treatment the composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally

occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method  
5 comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional PRTS, comprising administering to a patient in need of such treatment  
10 the composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group  
15 consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the  
20 polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the  
25 group consisting of SEQ ID NO:1-14, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test  
30 compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in  
35 altering expression of a target polynucleotide, wherein said target polynucleotide comprises a

sequence selected from the group consisting of SEQ ID NO:15-28, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

## BRIEF DESCRIPTION OF THE TABLES

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the present invention.

Table 2 shows the GenBank identification number and annotation of the nearest GenBank homolog for each polypeptide of the invention. The probability score for the match between each polypeptide and its GenBank homolog is also shown.

Table 3 shows structural features of each polypeptide sequence, including predicted motifs and domains, along with the methods, algorithms, and searchable databases used for analysis of each polypeptide.

Table 4 lists the cDNA and genomic DNA fragments which were used to assemble each

polynucleotide sequence, along with selected fragments of the polynucleotide sequences.

Table 5 shows the representative cDNA library for each polynucleotide of the invention.

Table 6 provides an appendix which describes the tissues and vectors used for construction of the cDNA libraries shown in Table 5.

5 Table 7 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

## DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood  
10 that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an,"  
15 and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings  
20 as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in  
25 connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

## DEFINITIONS

"PRTS" refers to the amino acid sequences of substantially purified PRTS obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and  
30 human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of PRTS. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of PRTS either by directly interacting with PRTS or by acting on components of the biological pathway in which PRTS participates.

An "allelic variant" is an alternative form of the gene encoding PRTS. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding PRTS include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as PRTS or a polypeptide with at least one functional characteristic of PRTS. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding PRTS, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding PRTS. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent PRTS. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of PRTS is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of PRTS. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of PRTS either by directly interacting with PRTS or by acting on components of the biological pathway in which PRTS

participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant.

Antibodies that bind PRTS polypeptides can be prepared using intact polypeptides or using fragments  
5 containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

10 The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to  
15 elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified  
20 sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or  
25 translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic PRTS, or of any oligopeptide thereof,  
30 to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding PRTS or fragments of PRTS may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (Applied Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
25	Asp	Asn, Glu
	Cys	Ala, Ser
	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
30	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
35	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
40	Val	Ile, Leu, Thr



Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

5 A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one  
10 biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

15 A "fragment" is a unique portion of PRTS or the polynucleotide encoding PRTS which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10,  
20 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the  
25 specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:15-28 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:15-28, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:15-28 is useful, for  
30 example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:15-28 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:15-28 and the region of SEQ ID NO:15-28 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-14 is encoded by a fragment of SEQ ID NO:15-28. A fragment  
35 of SEQ ID NO:1-14 comprises a region of unique amino acid sequence that specifically identifies

SEQ ID NO:1-14. For example, a fragment of SEQ ID NO:1-14 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-14. The precise length of a fragment of SEQ ID NO:1-14 and the region of SEQ ID NO:1-14 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full length" polynucleotide sequence encodes a "full length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to

compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Reward for match: 1*

5 *Penalty for mismatch: -2*

*Open Gap: 5 and Extension Gap: 2 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 11*

10 *Filter: on*

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such  
15 lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in  
20 a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment  
25 methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment  
30 program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

5 *Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 3*

*Filter: on*

10 Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length  
15 supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size and which contain all of the elements required for chromosome replication, segregation and maintenance.

20 The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific  
25 hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for  
30 annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of PRTS which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of

PRTS which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other  
5 chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of PRTS. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of PRTS.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide,  
10 polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably  
15 linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of  
20 amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

"Post-translational modification" of an PRTS may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the  
25 art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of PRTS.

"Probe" refers to nucleic acid sequences encoding PRTS, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical  
30 labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes. "Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

35 Probes and primers as used in the present invention typically comprise at least 15 contiguous

nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the  
5 specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR  
10 Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such  
15 purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of Texas South  
20 West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for  
25 microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences.  
30 Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to

those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the  
5 artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

10 Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs).  
15 Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and  
20 other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

25 The term "sample" is used in its broadest sense. A sample suspected of containing PRTS, nucleic acids encoding PRTS, or fragments thereof may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a  
30 protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will



reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed cells" includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 5 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may 10 possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides will generally have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in 15 which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 20 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

## THE INVENTION

25 The invention is based on the discovery of new human proteases (PRTS), the polynucleotides encoding PRTS, and the use of these compositions for the diagnosis, treatment, or prevention of gastrointestinal, cardiovascular, autoimmune/inflammatory, cell proliferative, developmental, epithelial, neurological, and reproductive disorders.

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide 30 sequences of the invention. Each polynucleotide and its corresponding polypeptide are correlated to a single Incyte project identification number (Incyte Project ID). Each polypeptide sequence is denoted by both a polypeptide sequence identification number (Polypeptide SEQ ID NO:) and an Incyte polypeptide sequence number (Incyte Polypeptide ID) as shown. Each polynucleotide sequence is denoted by both a polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and an 35 Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) as shown.

Table 2 shows sequences with homology to the polypeptides of the invention as identified by BLAST analysis against the GenBank protein (genpept) database. Columns 1 and 2 show the polypeptide sequence identification number (Polypeptide SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for each polypeptide of the invention. Column 3 shows the GenBank identification number (Genbank ID NO:) of the nearest GenBank homolog. Column 4 shows the probability score for the match between each polypeptide and its GenBank homolog. Column 5 shows the annotation of the GenBank homolog along with relevant citations where applicable, all of which are expressly incorporated by reference herein.

Table 3 shows various structural features of each of the polypeptides of the invention. Columns 1 and 2 show the polypeptide sequence identification number (SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for each polypeptide of the invention. Column 3 shows the number of amino acid residues in each polypeptide. Column 4 shows potential phosphorylation sites, and column 5 shows potential glycosylation sites, as determined by the MOTIFS program of the GCG sequence analysis software package (Genetics Computer Group, Madison WI). Column 6 shows amino acid residues comprising signature sequences, domains, and motifs. Column 7 shows analytical methods for protein structure/function analysis and in some cases, searchable databases to which the analytical methods were applied.

As shown in Table 4, the full length polynucleotide sequences of the present invention were assembled using cDNA sequences or coding (exon) sequences derived from genomic DNA, or any combination of these two types of sequences. Columns 1 and 2 list the polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and the corresponding Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) for each polynucleotide of the invention. Column 3 shows the length of each polynucleotide sequence in basepairs. Column 4 lists fragments of the polynucleotide sequences which are useful, for example, in hybridization or amplification technologies that identify SEQ ID NO:15-28 or that distinguish between SEQ ID NO:15-28 and related polynucleotide sequences. Column 5 shows identification numbers corresponding to cDNA sequences, coding sequences (exons) predicted from genomic DNA, and/or sequence assemblages comprised of both cDNA and genomic DNA. These sequences were used to assemble the full length polynucleotide sequences of the invention. Columns 6 and 7 of Table 4 show the nucleotide start (5') and stop (3') positions of the cDNA and genomic sequences in column 5 relative to their respective full length sequences.

The identification numbers in Column 5 of Table 4 may refer specifically, for example, to Incyte cDNAs along with their corresponding cDNA libraries. For example, 7032724H1 is the identification number of an Incyte cDNA sequence, and BRAXTDR12 is the cDNA library from which

it is derived. Incyte cDNAs for which cDNA libraries are not indicated were derived from pooled cDNA libraries (e.g., 70152356V1). Alternatively, the identification numbers in column 5 may refer to GenBank cDNAs or ESTs (e.g., g5364348) which contributed to the assembly of the full length polynucleotide sequences. Alternatively, the identification numbers in column 5 may refer to coding regions predicted by Genscan analysis of genomic DNA. For example, GNN.g6436155\_002.edit is the identification number of a Genscan-predicted coding sequence, with g6436155 being the GenBank identification number of the sequence to which Genscan was applied. The Genscan-predicted coding sequences may have been edited prior to assembly. (See Example IV.) Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an "exon stitching" algorithm. (See Example V.) Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an "exon-stretching" algorithm. (See Example V.) In some cases, Incyte cDNA coverage redundant with the sequence coverage shown in column 5 was obtained to confirm the final consensus polynucleotide sequence, but the relevant Incyte cDNA identification numbers are not shown.

Table 5 shows the representative cDNA libraries for those full length polynucleotide sequences which were assembled using Incyte cDNA sequences. The representative cDNA library is the Incyte cDNA library which is most frequently represented by the Incyte cDNA sequences which were used to assemble and confirm the above polynucleotide sequences. The tissues and vectors which were used to construct the cDNA libraries shown in Table 5 are described in Table 6.

The invention also encompasses PRTS variants. A preferred PRTS variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the PRTS amino acid sequence, and which contains at least one functional or structural characteristic of PRTS.

The invention also encompasses polynucleotides which encode PRTS. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28, which encodes PRTS. The polynucleotide sequences of SEQ ID NO:15-28, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding PRTS. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding PRTS. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:15-28 which has at

least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:15-28. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of PRTS.

5        It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding PRTS, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in  
10 accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring PRTS, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode PRTS and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring PRTS under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding PRTS or its  
15 derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding PRTS and its derivatives without altering the encoded amino acid sequences include the production of  
20 RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode PRTS and PRTS derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using  
25 reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding PRTS or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:15-28 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and  
30 S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of

DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Applied Biosystems), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is  
5 automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a  
10 variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding PRTS may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences,  
15 such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a  
20 known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown  
25 sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using  
30 commercially available software, such as OLIGO 4.06 primer analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full length cDNAs, it is preferable to use libraries that have been

size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

5        Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate  
10        software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

15        In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode PRTS may be cloned in recombinant DNA molecules that direct expression of PRTS, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express PRTS.

20        The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter PRTS-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites,  
25        alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

30        The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Cramer, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or  
35        improve the biological properties of PRTS, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial"

breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby  
5 maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding PRTS may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.) Alternatively,  
10 PRTS itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp.55-60; and Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Applied Biosystems). Additionally, the amino acid  
15 sequence of PRTS, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.)  
20 The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active PRTS, the nucleotide sequences encoding PRTS or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a  
25 suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding PRTS. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding PRTS. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where  
30 sequences encoding PRTS and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural  
35 and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate



for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding PRTS and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding PRTS. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; Engelhard, E.K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3224-3227; Sandig, V. et al. (1996) *Hum. Gene Ther.* 7:1937-1945; Takamatsu, N. (1987) *EMBO J.* 6:307-311; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) *Cancer Gen. Ther.* 5(6):350-356; Yu, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(13):6340-6344; Buller, R.M. et al. (1985) *Nature* 317(6040):813-815; McGregor, D.P. et al. (1994) *Mol. Immunol.* 31(3):219-226; and Verma, I.M. and N. Somia (1997) *Nature* 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding PRTS. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding PRTS can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPO11 plasmid (Life Technologies). Ligation of sequences encoding PRTS into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.*

264:5503-5509.) When large quantities of PRTS are needed, e.g. for the production of antibodies, vectors which direct high level expression of PRTS may be used. For example, vectors containing the strong, inducible SP6 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of PRTS. A number of vectors  
5 containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) *Methods Enzymol.* 153:516-544; and Scorer, C.A. et al. (1994)  
10 *Bio/Technology* 12:181-184.)

Plant systems may also be used for expression of PRTS. Transcription of sequences encoding PRTS may be driven by viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) *EMBO J.* 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be  
15 used. (See, e.g., Coruzzi, G. et al. (1984) *EMBO J.* 3:1671-1680; Broglie, R. et al. (1984) *Science* 224:838-843; and Winter, J. et al. (1991) *Results Probl. Cell Differ.* 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

20 In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding PRTS may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses PRTS in host cells. (See, e.g., Logan, J. and T. Shenk (1984) *Proc.*  
25 *Natl. Acad. Sci. USA* 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are  
30 constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of PRTS in cell lines is preferred. For example, sequences encoding PRTS can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous

expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk<sup>-</sup>* and *apr<sup>-</sup>* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding PRTS is inserted within a marker gene sequence, transformed cells containing sequences encoding PRTS can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding PRTS under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding PRTS and that express PRTS may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of PRTS using either

specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on PRTS is preferred, but a competitive binding  
5 assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and  
10 may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding PRTS include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding PRTS, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to  
15 synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates,  
20 cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding PRTS may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing  
25 polynucleotides which encode PRTS may be designed to contain signal sequences which direct secretion of PRTS through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation,  
30 lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing

of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding PRTS may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric PRTS protein containing a  
5 heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of PRTS activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and  
10 hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site  
15 located between the PRTS encoding sequence and the heterologous protein sequence, so that PRTS may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled PRTS may be achieved in  
20 vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, <sup>35</sup>S-methionine.

PRTS of the present invention or fragments thereof may be used to screen for compounds that  
25 specifically bind to PRTS. At least one and up to a plurality of test compounds may be screened for specific binding to PRTS. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of PRTS, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a  
30 natural binding partner. (See, e.g., Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which PRTS binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express PRTS, either as a secreted  
35 protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E.

coli. Cells expressing PRTS or cell membrane fractions which contain PRTS are then contacted with a test compound and binding, stimulation, or inhibition of activity of either PRTS or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with PRTS, either in solution or affixed to a solid support, and detecting the binding of PRTS to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

PRTS of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of PRTS. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for PRTS activity, wherein PRTS is combined with at least one test compound, and the activity of PRTS in the presence of a test compound is compared with the activity of PRTS in the absence of the test compound. A change in the activity of PRTS in the presence of the test compound is indicative of a compound that modulates the activity of PRTS. Alternatively, a test compound is combined with an in vitro or cell-free system comprising PRTS under conditions suitable for PRTS activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of PRTS may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding PRTS or their mammalian homologs may be “knocked out” in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent Number 5,175,383 and U.S. Patent Number 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the

resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding PRTS may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell  
5 lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding PRTS can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a  
10 polynucleotide encoding PRTS is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress PRTS, e.g., by secreting PRTS in its milk, may also serve as a convenient source  
15 of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

## THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of PRTS and proteases. In addition, the expression of PRTS is closely associated with gastrointestinal, epithelial, reproductive, cardiovascular, cancerous, and inflamed tissues, and  
20 with normal kidney and normal skin tissues. Therefore, PRTS appears to play a role in gastrointestinal, cardiovascular, autoimmune/inflammatory, cell proliferative, developmental, epithelial, neurological, and reproductive disorders. In the treatment of disorders associated with increased PRTS expression or activity, it is desirable to decrease the expression or activity of PRTS. In the treatment of disorders associated with decreased PRTS expression or activity, it is desirable to  
25 increase the expression or activity of PRTS.

Therefore, in one embodiment, PRTS or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PRTS. Examples of such disorders include, but are not limited to, a gastrointestinal disorder, such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma,  
30 dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-  
35 Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel

syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha<sub>1</sub>-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis

5    hepatitis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; a cardiovascular disorder, such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, and complications of thrombolysis, balloon

10   angioplasty, vascular replacement, and coronary artery bypass graft surgery, congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart

15   disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, congenital heart disease, and complications of cardiac transplantation; an autoimmune/inflammatory disorder, such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, atherosclerotic plaque rupture, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-

20   ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation,

25   osteoarthritis, degradation of articular cartilage, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a cell proliferative

30   disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract,



heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a developmental disorder, such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, bone resorption, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, age-related macular degeneration, and sensorineural hearing loss; an epithelial disorder, such as dyshidrotic eczema, allergic contact dermatitis, keratosis pilaris, melasma, vitiligo, actinic keratosis, basal cell carcinoma, squamous cell carcinoma, seborrheic keratosis, folliculitis, herpes simplex, herpes zoster, varicella, candidiasis, dermatophytosis, scabies, insect bites, cherry angioma, keloid, dermatofibroma, acrochordons, urticaria, transient acantholytic dermatosis, xerosis, eczema, atopic dermatitis, contact dermatitis, hand eczema, nummular eczema, lichen simplex chronicus, asteatotic eczema, stasis dermatitis and stasis ulceration, seborrheic dermatitis, psoriasis, lichen planus, pityriasis rosea, impetigo, ecthyma, dermatophytosis, tinea versicolor, warts, acne vulgaris, acne rosacea, pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, bullous pemphigoid, herpes gestationis, dermatitis herpetiformis, linear IgA disease, epidermolysis bullosa acquisita, dermatomyositis, lupus erythematosus, scleroderma and morphea, erythroderma, alopecia, figurate skin lesions, telangiectasias, hypopigmentation, hyperpigmentation, vesicles/bullae, exanthems, cutaneous drug reactions, papulonodular skin lesions, chronic non-healing wounds, photosensitivity diseases, epidermolysis bullosa simplex, epidermolytic hyperkeratosis, epidermolytic and nonepidermolytic palmoplantar keratoderma, ichthyosis bullosa of Siemens, ichthyosis exfoliativa, keratosis palmaris et plantaris, keratosis palmoplantaris, palmoplantar keratoderma, keratosis punctata, Meesmann's corneal dystrophy, pachyonychia congenita, white sponge nevus, steatocystoma multiplex, epidermal nevi/epidermolytic hyperkeratosis type, monilethrix, trichothiodystrophy, chronic hepatitis/cryptogenic cirrhosis, and colorectal hyperplasia; a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial

insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system including Down syndrome, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; and a reproductive disorder, such as infertility, including tubal disease, ovulatory defects, and endometriosis, a disorder of prolactin production, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia.

In another embodiment, a vector capable of expressing PRTS or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PRTS including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified PRTS in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PRTS including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of PRTS may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of PRTS including, but not limited to, those listed above.

In a further embodiment, an antagonist of PRTS may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of PRTS. Examples of such disorders include, but are not limited to, those gastrointestinal, cardiovascular, autoimmune/inflammatory, cell proliferative, developmental, epithelial, neurological, and reproductive disorders described above. In one aspect, an antibody which specifically binds PRTS may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express PRTS.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding PRTS may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of PRTS including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of PRTS may be produced using methods which are generally known in the art. In particular, purified PRTS may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind PRTS. Antibodies to PRTS may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with PRTS or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to PRTS have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of PRTS amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to PRTS may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J.

Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce PRTS-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for PRTS may also be generated. For example, such fragments include, but are not limited to,  $F(ab')_2$  fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the  $F(ab')_2$  fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between PRTS and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering PRTS epitopes is generally used, but a competitive binding assay may also be employed (Pound, *supra*).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for PRTS. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of PRTS-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple PRTS epitopes, represents the average affinity, or avidity, of the antibodies for PRTS. The  $K_a$  determined for

a preparation of monoclonal antibodies, which are monospecific for a particular PRTS epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the PRTS-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of PRTS, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of PRTS-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding PRTS, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding PRTS. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding PRTS. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc., Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, supra; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding PRTS may be used for

somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) Science 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) Science 270:475-480; Bordignon, C. et al. (1995) Science 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) Cell 75:207-216; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:643-666; Crystal, R.G. et al. (1995) Hum. Gene Therapy 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) Science 270:404-410; Verma, I.M. and N. Somia (1997) Nature 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) Nature 335:395-396; Poeschla, E. et al. (1996) Proc. Natl. Acad. Sci. USA. 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in PRTS expression or regulation causes disease, the expression of PRTS from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in PRTS are treated by constructing mammalian expression vectors encoding PRTS and introducing these vectors by mechanical means into PRTS-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii) ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) Annu. Rev. Biochem. 62:191-217; Ivics, Z. (1997) Cell 91:501-510; Boulay, J-L. and H. Récipon (1998) Curr. Opin. Biotechnol. 9:445-450).

Expression vectors that may be effective for the expression of PRTS include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). PRTS may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or  $\beta$ -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) Proc. Natl. Acad. Sci. USA 89:5547-5551; Gossen, M. et al. (1995) Science 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998)

Curr. Opin. Biotechnol. 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and Blau, H.M. *supra*), or (iii) a tissue-specific promoter or the native promoter of the endogenous  
5 gene encoding PRTS from a normal individual.

Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method  
10 (Graham, F.L. and A.J. Eb (1973) Virology 52:456-467), or by electroporation (Neumann, E. et al. (1982) EMBO J. 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to PRTS expression are treated by constructing a retrovirus vector consisting of (i) the  
15 polynucleotide encoding PRTS under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) Proc.  
20 Natl. Acad. Sci. USA 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al. (1987) J. Virol. 61:1647-1650; Bender, M.A. et al. (1987) J. Virol. 61:1639-1646; Adam, M.A. and A.D. Miller (1988) J. Virol. 62:3802-3806; Dull, T. et al. (1998) J. Virol. 72:8463-8471; Zufferey, R.  
25 et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4<sup>+</sup> T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene  
30 therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding PRTS to cells which have one or more genetic abnormalities with respect to

the expression of PRTS. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) *Transplantation* 27:263-268). Potentially useful adenoviral vectors are  
5 described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) *Annu. Rev. Nutr.* 19:511-544 and Verma, I.M. and N. Somia (1997) *Nature* 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver  
10 polynucleotides encoding PRTS to target cells which have one or more genetic abnormalities with respect to the expression of PRTS. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing PRTS to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has  
15 been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) *Exp. Eye Res.* 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under  
20 the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) *J. Virol.* 73:519-532 and Xu, H. et al. (1994) *Dev. Biol.* 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids  
25 containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding PRTS to target cells. The biology of the prototypic alphavirus,  
30 Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) *Curr. Opin. Biotechnol.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g.,



protease and polymerase). Similarly, inserting the coding sequence for PRTS into the alphavirus genome in place of the capsid-coding region results in the production of a large number of PRTS-coding RNAs and the synthesis of high levels of PRTS in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) Virology 228:74-83). The wide host range of alphaviruses will allow the introduction of PRTS into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding PRTS.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for

chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis.

Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding PRTS. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize  
5 complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be  
10 extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding PRTS. Compounds  
15 which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of  
20 polynucleotide expression. Thus, in the treatment of disorders associated with increased PRTS expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding PRTS may be therapeutically useful, and in the treatment of disorders associated with decreased PRTS expression or activity, a compound which specifically promotes expression of the polynucleotide encoding PRTS may be therapeutically useful.

25 At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound  
30 based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding PRTS is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding PRTS are assayed by  
35 any method commonly known in the art. Typically, the expression of a specific nucleotide is detected

by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding PRTS. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruice, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruice, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of PRTS, antibodies to PRTS, and mimetics, agonists, antagonists, or inhibitors of PRTS.

The compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting

formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle  
5 injection, and obviates the need for potentially toxic penetration enhancers.

Compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

Specialized forms of compositions may be prepared for direct intracellular delivery of  
10 macromolecules comprising PRTS or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, PRTS or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et  
15 al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for  
20 administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example PRTS or fragments thereof, antibodies of PRTS, and agonists, antagonists or inhibitors of PRTS, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by  
25 calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is  
30 preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active

moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

### DIAGNOSTICS

In another embodiment, antibodies which specifically bind PRTS may be used for the diagnosis of disorders characterized by expression of PRTS, or in assays to monitor patients being treated with PRTS or agonists, antagonists, or inhibitors of PRTS. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for PRTS include methods which utilize the antibody and a label to detect PRTS in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring PRTS, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of PRTS expression. Normal or standard values for PRTS expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibodies to PRTS under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of PRTS expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding PRTS may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of PRTS may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of PRTS, and to monitor regulation of PRTS levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding PRTS or closely related molecules may be used to identify nucleic acid sequences which encode PRTS. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding PRTS, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the PRTS encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:15-28 or from genomic sequences including promoters, enhancers, and introns of the PRTS gene.

Means for producing specific hybridization probes for DNAs encoding PRTS include the cloning of polynucleotide sequences encoding PRTS or PRTS derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding PRTS may be used for the diagnosis of disorders associated with expression of PRTS. Examples of such disorders include, but are not limited to, a gastrointestinal disorder, such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease,  $\alpha_1$ -antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; a cardiovascular disorder, such as arteriovenous fistula,

atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, and complications of thrombolysis, balloon angioplasty, vascular replacement, and coronary artery bypass graft surgery, congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease,

5 degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, congenital heart disease, and complications of cardiac transplantation; an

10 autoimmune/inflammatory disorder, such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, atherosclerotic plaque rupture, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus,

15 emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, degradation of articular cartilage, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's

20 syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal

25 hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a developmental disorder,

30 such as renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, bone resorption, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism,

hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spina bifida, anencephaly, craniorachischisis, congenital glaucoma, cataract, age-related macular degeneration, and sensorineural hearing loss; an epithelial disorder, such as dyshidrotic eczema, allergic contact dermatitis, keratosis pilaris, melasma, vitiligo, actinic keratosis, basal cell carcinoma, squamous cell carcinoma, seborrheic keratosis, folliculitis, herpes simplex, herpes zoster, varicella, candidiasis, dermatophytosis, scabies, insect bites, cherry angioma, keloid, dermatofibroma, acrochordons, urticaria, transient acantholytic dermatosis, xerosis, eczema, atopic dermatitis, contact dermatitis, hand eczema, nummular eczema, lichen simplex chronicus, asteatotic eczema, stasis dermatitis and stasis ulceration, seborrheic dermatitis, psoriasis, lichen planus, pityriasis rosea, impetigo, ecthyma, dermatophytosis, tinea versicolor, warts, acne vulgaris, acne rosacea, pemphigus vulgaris, pemphigus foliaceus, paraneoplastic pemphigus, bullous pemphigoid, herpes gestationis, dermatitis herpetiformis, linear IgA disease, epidermolysis bullosa acquisita, dermatomyositis, lupus erythematosus, scleroderma and morphea, erythroderma, alopecia, figurate skin lesions, telangiectasias, hypopigmentation, hyperpigmentation, vesicles/bullae, exanthems, cutaneous drug reactions, papulonodular skin lesions, chronic non-healing wounds, photosensitivity diseases, epidermolysis bullosa simplex, epidermolytic hyperkeratosis, epidermolytic and nonepidermolytic palmoplantar keratoderma, ichthyosis bullosa of Siemens, ichthyosis exfoliativa, keratosis palmaris et plantaris, keratosis palmoplantaris, palmoplantar keratoderma, keratosis punctata, Meesmann's corneal dystrophy, pachyonychia congenita, white sponge nevus, steatocystoma multiplex, epidermal nevi/epidermolytic hyperkeratosis type, monilethrix, trichothiodystrophy, chronic hepatitis/cryptogenic cirrhosis, and colorectal hyperplasia; a neurological disorder, such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system including Down syndrome, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic



disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; and a reproductive disorder, such as infertility, including tubal disease, ovulatory defects, and endometriosis, a disorder of  
5 prolactin production, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease,  
10 impotence, carcinoma of the male breast, and gynecomastia. The polynucleotide sequences encoding PRTS may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered PRTS expression. Such qualitative or quantitative methods are well known in the art.

15 In a particular aspect, the nucleotide sequences encoding PRTS may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding PRTS may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard  
20 value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding PRTS in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

25 In order to provide a basis for the diagnosis of a disorder associated with expression of PRTS, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding PRTS, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with  
30 values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated,

hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

5       With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further  
10       progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding PRTS may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding PRTS, or a fragment of a polynucleotide complementary to the polynucleotide encoding PRTS, and will be  
15       employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding PRTS may be used to detect single nucleotide polymorphisms (SNPs). SNPs are  
20       substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP, oligonucleotide primers derived from the polynucleotide sequences encoding PRTS are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal  
25       tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP  
30       (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high

throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of PRTS include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described below. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, PRTS, fragments of PRTS, or antibodies specific for PRTS may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies,

or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and

analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are  
5 visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein  
10 spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

15 A proteomic profile may also be generated using antibodies specific for PRTS to quantify the levels of PRTS expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoz, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of  
20 methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J.  
25 Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological  
30 sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the

individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding PRTS may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, for example, Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding PRTS on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as

linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, PRTS, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between PRTS and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with PRTS, or fragments thereof, and washed. Bound PRTS is then detected by methods well known in the art. Purified PRTS can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding PRTS specifically compete with a test compound for binding PRTS. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with PRTS.

In additional embodiments, the nucleotide sequences which encode PRTS may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications and publications, mentioned above and below, in

particular U.S. Ser. No. 60/172,055, U.S. Ser. No. 60/177,334, U.S. Ser. No. 60/178,884, and U.S. Ser. No. 60/179,903, are expressly incorporated by reference herein.

## EXAMPLES

### 5 I. Construction of cDNA Libraries

Incyte cDNAs were derived from cDNA libraries described in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA) and shown in Table 4, column 5. The Incyte cDNAs shown for SEQ ID NO:15 were derived from cDNA libraries constructed from small intestine, ovary, lung, skin, breast, prostate epithelium, and mixed myometrial tissues; umbilical cord blood, and teratocarcinoma cells  
10 which contained neuronal precursors. The Incyte cDNAs shown for SEQ ID NO:17 were derived from cDNA libraries constructed from a bronchial epithelium primary cell line, dermal microvascular endothelial cells, pancreas, ileum tissue associated with Crohn's disease, rib bone tissue associated with Patau's syndrome, kidney, thoracic dorsal root ganglion, and penis corpus cavernosum tissue. The Incyte cDNA shown for SEQ ID NO:18 was derived from a cDNA library constructed from brain  
15 tumor tissue. The Incyte cDNAs shown for SEQ ID NO:19 were derived from cDNA libraries constructed from adrenal gland, colon, and breast tissue. The Incyte cDNAs shown for SEQ ID NO:20 were derived from cDNA libraries constructed from T-lymphocytes, lung, breast, and penis corpus cavernosum tissues. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL  
20 (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A)+ RNA was isolated  
25 using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA  
30 libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the



appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g.,

- 5 PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), or pINCY (Incyte Genomics, Palo Alto CA), or derivatives thereof. Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## 10 II. Isolation of cDNA Clones

- Plasmids obtained as described in Example I were recovered from host cells by *in vivo* excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 15 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

- Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal 20 cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

## III. Sequencing and Analysis

- 25 Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Applied Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared 30 using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI 35 protocols and base calling software; or other sequence analysis systems known in the art. Reading

frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VIII.

The polynucleotide sequences derived from Incyte cDNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations thereof were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. (HMM is a probabilistic approach which analyzes consensus primary structures of gene families. See, for example, Eddy, S.R. (1996) *Curr. Opin. Struct. Biol.* 6:361-365.) The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were assembled to produce full length polynucleotide sequences. Alternatively, GenBank cDNAs, GenBank ESTs, stitched sequences, stretched sequences, or Genscan-predicted coding sequences (see Examples IV and V) were used to extend Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Alternatively, a polypeptide of the invention may begin at any of the methionine residues of the full length translated polypeptide. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the GenBank protein databases (genpept), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 7 summarizes the tools, programs, and algorithms used for the analysis and assembly of Incyte cDNA and full length sequences and provides applicable descriptions, references, and threshold parameters. The first column of Table 7 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match

between two sequences (the higher the score or the lower the probability value, the greater the identity between two sequences).

The programs described above for the assembly and analysis of full length polynucleotide and polypeptide sequences were also used to identify polynucleotide sequence fragments from SEQ ID

- 5 NO:15-28. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies are described in Table 4, column 4.

#### IV. Identification and Editing of Coding Sequences from Genomic DNA

- Putative proteases were initially identified by running the Genscan gene identification program against public genomic sequence databases (e.g., gbpri and gbhtg). Genscan is a general-purpose gene  
10 identification program which analyzes genomic DNA sequences from a variety of organisms (See Burge, C. and S. Karlin (1997) J. Mol. Biol. 268:78-94, and Burge, C. and S. Karlin (1998) Curr. Opin. Struct. Biol. 8:346-354). The program concatenates predicted exons to form an assembled cDNA sequence extending from a methionine to a stop codon. The output of Genscan is a FASTA database of polynucleotide and polypeptide sequences. The maximum range of sequence for Genscan  
15 to analyze at once was set to 30 kb. To determine which of these Genscan predicted cDNA sequences encode proteases, the encoded polypeptides were analyzed by querying against PFAM models for proteases. Potential proteases were also identified by homology to Incyte cDNA sequences that had been annotated as proteases. These selected Genscan-predicted sequences were then compared by BLAST analysis to the genpept and gbpri public databases. Where necessary, the Genscan-predicted  
20 sequences were then edited by comparison to the top BLAST hit from genpept to correct errors in the sequence predicted by Genscan, such as extra or omitted exons. BLAST analysis was also used to find any Incyte cDNA or public cDNA coverage of the Genscan-predicted sequences, thus providing evidence for transcription. When Incyte cDNA coverage was available, this information was used to correct or confirm the Genscan predicted sequence. Full length polynucleotide sequences were obtained  
25 by assembling Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the assembly process described in Example III. Alternatively, full length polynucleotide sequences were derived entirely from edited or unedited Genscan-predicted coding sequences.

#### V. Assembly of Genomic Sequence Data with cDNA Sequence Data

##### 30 "Stitched" Sequences

Partial cDNA sequences were extended with exons predicted by the Genscan gene identification program described in Example IV. Partial cDNAs assembled as described in Example III were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm based on graph theory

and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or extended to create a full length sequence.

Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified, and intervals thus identified were considered to be equivalent by transitivity.

5 For example, if an interval was present on a cDNA and two genomic sequences, then all three intervals were considered to be equivalent. This process allows unrelated but consecutive genomic sequences to be brought together, bridged by cDNA sequence. Intervals thus identified were then "stitched" together by the stitching algorithm in the order that they appear along their parent sequences to generate the longest possible sequence, as well as sequence variants. Linkages between intervals which proceed  
10 along one type of parent sequence (cDNA to cDNA or genomic sequence to genomic sequence) were given preference over linkages which change parent type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbpri public databases. Incorrect exons predicted by Genscan were corrected by comparison to the top BLAST hit from genpept. Sequences were further extended with additional cDNA sequences, or by inspection of  
15 genomic DNA, when necessary.

#### "Stretched" Sequences

Partial DNA sequences were extended to full length with an algorithm based on BLAST analysis. First, partial cDNAs assembled as described in Example III were queried against public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases using  
20 the BLAST program. The nearest GenBank protein homolog was then compared by BLAST analysis to either Incyte cDNA sequences or GenScan exon predicted sequences described in Example IV. A chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. Insertions or deletions may occur in the chimeric protein with respect to the original GenBank protein homolog. The GenBank protein homolog,  
25 the chimeric protein, or both were used as probes to search for homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore "stretched" or extended by the addition of homologous genomic sequences. The resultant stretched sequences were examined to determine whether it contained a complete gene.

#### **VI. Chromosomal Mapping of PRTS Encoding Polynucleotides**

30 The sequences which were used to assemble SEQ ID NO:15-28 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:15-28 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 7). Radiation hybrid and genetic mapping data available  
35 from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for

Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

Map locations are represented by ranges, or intervals, or human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

## VII. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel (1995) *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum} \{ \text{length}(\text{Seq. 1}), \text{length}(\text{Seq. 2}) \}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For

example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

5 Alternatively, polynucleotide sequences encoding PRTS are analyzed with respect to the tissue sources from which they were derived. For example, some full length sequences are assembled, at least in part, with overlapping Incyte cDNA sequences (see Example III). Each cDNA sequence is derived from a cDNA library constructed from a human tissue. Each human tissue is classified into one of the following organ/tissue categories: cardiovascular system; connective tissue; digestive system;  
10 embryonic structures; endocrine system; exocrine glands; genitalia, female; genitalia, male; germ cells; hemic and immune system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs; skin; stomatognathic system; unclassified/mixed; or urinary tract. The number of libraries in each category is counted and divided by the total number of libraries across all categories. Similarly, each human tissue is classified into one of the following disease/condition categories: cancer,  
15 cell line, developmental, inflammation, neurological, trauma, cardiovascular, pooled, and other, and the number of libraries in each category is counted and divided by the total number of libraries across all categories. The resulting percentages reflect the tissue- and disease-specific expression of cDNA encoding PRTS. cDNA sequences and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA).

#### 20 **VIII. Extension of PRTS Encoding Polynucleotides**

Full length polynucleotide sequences were also produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer was synthesized to initiate 3' extension of the known fragment. The initial primers were designed using  
25 OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension  
30 was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and 2-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme

(Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100 µl PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5 µl of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 µl to 10 µl aliquot of the reaction mixture was analyzed by electrophoresis on a 1% agarose gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, full length polynucleotide sequences are verified using the above procedure or are used to obtain 5' regulatory sequences using the above procedure along with oligonucleotides

designed for such extension, and an appropriate genomic library.

#### **IX. Labeling and Use of Individual Hybridization Probes**

Hybridization probes derived from SEQ ID NO:15-28 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

#### **X. Microarrays**

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, *supra.*), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Schna (1999), *supra.*). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schna, M. et al. (1995) *Science* 270:467-470; Shalon, D. et al. (1996) *Genome Res.* 6:639-645; Marshall, A. and J. Hodgson (1998) *Nat. Biotechnol.* 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array



elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

#### **Tissue or Cell Sample Preparation**

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)<sup>+</sup> RNA is purified using the oligo-(dT) cellulose method. Each poly(A)<sup>+</sup> RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ $\mu$ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ $\mu$ l RNase inhibitor, 500  $\mu$ M dATP, 500  $\mu$ M dGTP, 500  $\mu$ M dTTP, 40  $\mu$ M dCTP, 40  $\mu$ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)<sup>+</sup> RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)<sup>+</sup> RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14  $\mu$ l 5X SSC/0.2% SDS.

#### **Microarray Preparation**

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5  $\mu$ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and

coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US Patent No. 5,807,522, incorporated herein by reference. 1 µl of the array element DNA, at an average concentration of 100 ng/µl, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60°C followed by washes in 0.2% SDS and distilled water as before.

### **Hybridization**

Hybridization reactions contain 9 µl of sample mixture consisting of 0.2 µg each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65°C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm<sup>2</sup> coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 µl of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60°C. The arrays are washed for 10 min at 45°C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45°C in a second wash buffer (0.1X SSC), and dried.

### **Detection**

Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

#### **XI. Complementary Polynucleotides**

Sequences complementary to the PRTS-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring PRTS. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of PRTS. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the PRTS-encoding transcript.

#### **XII. Expression of PRTS**

Expression and purification of PRTS is achieved using bacterial or virus-based expression systems. For expression of PRTS in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element.

Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic

resistant bacteria express PRTS upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of PRTS in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding PRTS  
5 by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (Sec Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA  
10 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, PRTS is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized  
15 glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from PRTS at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins  
20 (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified PRTS obtained by these methods can be used directly in the assays shown in Examples XVI, XVII, XVIII, and XIX, where applicable.

### XIII. Functional Assays

PRTS function is assessed by expressing the sequences encoding PRTS at physiologically  
25 elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include PCMV SPORT (Life Technologies) and PCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome  
30 formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-

based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of PRTS on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding PRTS and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding PRTS and other genes of interest can be analyzed by northern analysis or microarray techniques.

#### **XIV. Production of PRTS Specific Antibodies**

PRTS substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the PRTS amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using FMOC chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for anti-peptide and anti-PRTS activity by, for example, binding the peptide or PRTS to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

**XV. Purification of Naturally Occurring PRTS Using Specific Antibodies**

Naturally occurring or recombinant PRTS is substantially purified by immunoaffinity chromatography using antibodies specific for PRTS. An immunoaffinity column is constructed by covalently coupling anti-PRTS antibody to an activated chromatographic resin, such as CNBr-activated  
5 SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing PRTS are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of PRTS (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/PRTS binding  
10 (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and PRTS is collected.

**XVI. Identification of Molecules Which Interact with PRTS**

PRTS, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules  
15 previously arrayed in the wells of a multi-well plate are incubated with the labeled PRTS, washed, and any wells with labeled PRTS complex are assayed. Data obtained using different concentrations of PRTS are used to calculate values for the number, affinity, and association of PRTS with the candidate molecules.

Alternatively, molecules interacting with PRTS are analyzed using the yeast two-hybrid  
20 system as described in Fields, S. and O. Song (1989) *Nature* 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

PRTS may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent  
25 No. 6,057,101).

**XVII. Demonstration of PRTS Activity**

Protease activity is measured by the hydrolysis of appropriate synthetic peptide substrates conjugated with various chromogenic molecules in which the degree of hydrolysis is quantified by spectrophotometric (or fluorometric) absorption of the released chromophore (Beynon, R.J. and J.S.  
30 Bond (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York NY, pp.25-55). Peptide substrates are designed according to the category of protease activity as endopeptidase (serine, cysteine, aspartic proteases, or metalloproteases), aminopeptidase (leucine aminopeptidase), or carboxypeptidase (carboxypeptidases A and B, procollagen C-proteinase). Commonly used chromogens are 2-naphthylamine, 4-nitroaniline, and furylacrylic acid. Assays are

performed at ambient temperature and contain an aliquot of the enzyme and the appropriate substrate in a suitable buffer. Reactions are carried out in an optical cuvette, and the increase/decrease in absorbance of the chromogen released during hydrolysis of the peptide substrate is measured. The change in absorbance is proportional to the enzyme activity in the assay.

5        An alternate assay for ubiquitin hydrolase activity measures the hydrolysis of a ubiquitin precursor. The assay is performed at ambient temperature and contains an aliquot of PRTS and the appropriate substrate in a suitable buffer. Chemically synthesized human ubiquitin-valine may be used as substrate. Cleavage of the C-terminal valine residue from the substrate is monitored by capillary electrophoresis (Franklin, K. et al. (1997) *Anal. Biochem.* 247:305-309).

10        In the alternative, an assay for protease activity takes advantage of fluorescence resonance energy transfer (FRET) that occurs when one donor and one acceptor fluorophore with an appropriate spectral overlap are in close proximity. A flexible peptide linker containing a cleavage site specific for PRTS is fused between a red-shifted variant (RSGFP4) and a blue variant (BFP5) of Green Fluorescent Protein. This fusion protein has spectral properties that suggest energy transfer is occurring from BFP5  
15        to RSGFP4. When the fusion protein is incubated with PRTS, the substrate is cleaved, and the two fluorescent proteins dissociate. This is accompanied by a marked decrease in energy transfer which is quantified by comparing the emission spectra before and after the addition of PRTS (Mitra, R.D. et al. (1996) *Gene* 173:13-17). This assay can also be performed in living cells. In this case the fluorescent substrate protein is expressed constitutively in cells and PRTS is introduced on an inducible vector so  
20        that FRET can be monitored in the presence and absence of PRTS (Sagot, I. et al. (1999) *FEBS Lett.* 447:53-57).

#### **XVIII. Identification of PRTS Substrates**

Phage display libraries can be used to identify optimal substrate sequences for PRTS. A random hexamer followed by a linker and a known antibody epitope is cloned as an N-terminal  
25        extension of gene III in a filamentous phage library. Gene III codes for a coat protein, and the epitope will be displayed on the surface of each phage particle. The library is incubated with PRTS under proteolytic conditions so that the epitope will be removed if the hexamer codes for a PRTS cleavage site. An antibody that recognizes the epitope is added along with immobilized protein A. Uncleaved phage, which still bear the epitope, are removed by centrifugation. Phage in the supernatant are then  
30        amplified and undergo several more rounds of screening. Individual phage clones are then isolated and sequenced. Reaction kinetics for these peptide substrates can be studied using an assay in Example XVII, and an optimal cleavage sequence can be derived (Ke, S.H. et al. (1997) *J. Biol. Chem.* 272:16603-16609).

To screen for in vivo PRTS substrates, this method can be expanded to screen a cDNA

expression library displayed on the surface of phage particles (T7SELECT™10-3 Phage display vector, Novagen, Madison, WI) or yeast cells (pYD1 yeast display vector kit, Invitrogen, Carlsbad, CA). In this case, entire cDNAs are fused between Gene III and the appropriate epitope.

#### **XIX. Identification of PRTS Inhibitors**

5           Compounds to be tested are arrayed in the wells of a multi-well plate in varying concentrations along with an appropriate buffer and substrate, as described in the assays in Example XVII. PRTS activity is measured for each well and the ability of each compound to inhibit PRTS activity can be determined, as well as the dose-response kinetics. This assay could also be used to identify molecules which enhance PRTS activity.

10           In the alternative, phage display libraries can be used to screen for peptide PRTS inhibitors. Candidates are found among peptides which bind tightly to a protease. In this case, multi-well plate wells are coated with PRTS and incubated with a random peptide phage display library or a cyclic peptide library (Koivunen, E. et al. (1999) Nat. Biotechnol. 17:768-774). Unbound phage are washed away and selected phage amplified and rescreened for several more rounds. Candidates are tested for  
15 PRTS inhibitory activity using an assay described in Example XVII.

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be  
20 understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.



Table 1

Incyte Project ID	Polypeptide SEQ ID NO:	Incyte Polypeptide ID	Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID
1714846	1	1714846CD1	15	1714846CB1
1856589	2	1856589CD1	16	1856589CB1
2617672	3	2617672CD1	17	2617672CB1
2769104	4	2769104CD1	18	2769104CB1
4802789	5	4802789CD1	19	4802789CB1
60116897	6	60116897CD1	20	60116897CB1
1866356	7	1866356CD1	21	1866356CB1
1872095	8	1872095CD1	22	1872095CB1
2278688	9	2278688CD1	23	2278688CB1
4043361	10	4043361CD1	24	4043361CB1
3937958	11	3937958CD1	25	3937958CB1
7257324	12	7257324CD1	26	7257324CB1
7472038	13	7472038CD1	27	7472038CB1
7472041	14	7472041CD1	28	7472041CB1

Table 2

Polypeptide SEQ ID NO:	Incyte Polypeptide ID	GenBank ID NO:	Probability Score	GenBank Homolog
1	1714846	g6941890	0.0	Ubiquitin-specific processing protease [Mus musculus] (Valero, R. et al. (1999) Genomics 62:395-405)
2	1856589	g1143194	1.2e-45	Prostasin [Homo sapiens] (Yu, J.X. et al. (1994) J. Biol. Chem. 269:18843-18848)
3	2617672	g4929827	8.0e-118	Tubulo-interstitial nephritis antigen TIN-Ag [Mus musculus]
4	2769104	g179644	3.3e-28	Human complement C1r [Homo sapiens]
5	4802789	g4454565	4.1e-30	Ubiquitin processing protease [Homo sapiens] (Cai, S. et al. Proc. Natl. Acad. Sci. USA (1999) 96:2828-2833)
6	60116897	g9886747	0.0	VEGF induced aminopeptidase [Mus musculus]
7	1866356CD1	g2088823	1.5e-68	Similarity to the peptidase family A2 [Caenorhabditis elegans]
8	1872095CD1	g2347100	1.7e-22	Ubiquitin-specific protease [Arabidopsis thaliana]
9	2278688CD1	g1184161	0.0	Aminopeptidase [Mus musculus]
10	4043361CD1	g9843781	2.6e-104	Putative pyroglutaryl-peptidase I [Mus musculus]
11	3937958CD1	g180950	5.7e-16	Carboxylesterase [Homo sapiens]
12	7257324CD1	g2116650	1.1e-78	Alpha-1-antitrypsin [Cercopithecus aethiops] (Colau, B. et al. (1984) DNA 3:327-330; Yoshida, K. et al. (1999) J. Biochem. Mol. Biol. Biophys. 3:59-63)
13	7472038CD1	g293230	4.0e-106	Aspartic protease [Aedes aegypti]
14	7472041CD1	g3088553	1.1e-14	Cystatin-related epididymal spermatogenic protein [Homo sapiens] (Cornwall, G.A., Hsia, N., and Sutton, H.G. Biochem. J. (1999) 340(Pt 1):85-93)

Table 3

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
1	1714846CD1	1055	S85 T95 S109 T123 S136 T147 T286 S357 S375 S467 S489 T541 S546 T557 S631 S632 S745 T796 T824 S835 S892 S945 S1021 S1032 T1050 T55 S113 T235 T267 S354 S460 S513 S582 S719 Y575 Y872 Y873	N31 N256 N561 N646 N833	Ubiquitin C-terminal hydrolase: L232-W703, K823-F899 Ubiquitin C-term. hydrolase signature 1: V169-Y200 Ubiquitin C-term. hydrolase signature 2: G170-L187, S258-T267, P590-D614, E617-R638, I587-N656, Y591-Y608, N173-N408, D562-G601	HMER-PFAM BLIMPS- BLOCKS BLAST- PRODOM BLAST-DOMO MOTIFS
2	1856589CD1	358	S47 T188 T5 S105 T143 Y247	N150	Chymotrypsin family: G115-C130, F173-V187, E277-A289 Trypsin family: W100-I327, C114-C130, N278-V301, P314-I327, W100-M331 Trypsin family His active site: V125-C130, L106-N150 Trypsin family Ser active site: V265-K310 Kringles domain: C114-S131, I196-S217, G286-I327 Apple domain: G116-P148, V187-Q221, I270-W304, E305-N333	HMER-PFAM BLIMPS- BLOCKS BLIMPS- PRINTS ProfileScan BLAST- PRODOM BLAST-DOMO MOTIFS
3	2617672CD1	467	T80 T117 T126 T169 T205 S296 T411 T180 S210 S239 S401 T417	N78 N161	Signal peptide: M1-A19, M1-G21 Papain family protease: D222-W456, Q223-F232, Q267-L275, T399-G408, Y420-H436, Q223-A238, H400-E410, Y420-S426, D222-R441, F76-G457, D145-V455 Cys protease His active site: G398-G408 Tubulointerstitial nephritis antigen: G45-I193	HMER SPScan HMER-PFAM BLIMPS- BLOCKS BLIMPS- PRINTS BLAST- PRODOM BLAST-DOMO MOTIFS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
4	2769104CD1	187	S67 T162 S131 S134 T138	N147	CUB domain (extracellular domain found in complement proteins): G40-Y160 Complement C1r/C1s repeat: C36-V163, Q51-Y160, M24-Y160 Signal peptide: M1-A35	HMME-PFAM BLAST-DOMO MOTIFS
5	4802789CD1	289	T18 S28 S109 T213 S236 S261 S17 S102 S108 S188 S225 T265 S271	N119 N186	Transmembrane domain: W25-L52 Ubiquitin C-term. hydrolase signature 1: G191-L208	SPScan HMME HMME
6	60116897CD1	960	S225 S483 T57 T87 S124 T197 S321 T343 S357 T407 S502 S607 S701 S738 S744 S817 S906 S926 T933 S10 S94 T183 S221 T256 S303 S359 S432 S486 S558 S740 S781 T830 T951 Y312 Y622 Y679 Y885	N85 N103 N119 N219 N294 N405 N431 N650 N714 N879	Signal peptide: M1-A44 Zn metalloproteinase family M1: L69-G458 Zn membrane alanyl dipeptidase: R205-F220, F253-V268, F331-L341, V367-T382, W386-Y398 Neutral Zn-protease: W64-S500, G529-L837, T521-S899, W64-T902, P54-D555, K553-L837, V849-L956 Neutral Zn-protease, Zn binding region: V367-W376, V367-F377	SPScan HMME-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODROM BLAST-DOMO MOTIFS
7	1866356CD1	525	S82 S90 T159 T174 S288 S290 T311 T356 S397 T479 S522 S107 S122 S165 S228		Signal peptide: M1-C35 Signal peptide: M1-S26 Similarity to the peptidase family A2 PD138963: F157-G422	SPScan SPScan BLAST-PRODROM

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
8	1872095CD1	795	S274 S279 S522 Y523 T693 T251 S274 S314 S332 T337 S377 S378 S383 S392 S470 T472 S555 S557 S580 T582 T619 S620 T621	N171 N381 N443 N448 N536 N617 N670 N436 N711 N712 N720 N788	Ubiquitin carboxyl-terminal hydrolase 1 motif: G199-I213 Ubiquitin carboxyl-terminal hydrolase 2 motif: Y593-H610 Ubiquitin carboxyl-terminal hydrolases family UCH-1: T198-L229 Ubiquitin carboxyl-terminal hydrolases family UCH-2: K589-K701 Protease, ubiquitin hydrolase, ubiquitin-specific enzyme, deubiquitinating carboxyl-terminal thiolesterase, processing, conjugation: PD017412: S470-L541 Ubiquitin carboxyl-terminal hydrolases family 2: DM00659 P40818 782-1103: L203-D386	MOTIFS MOTIFS HMMER-PFAM HMMER-PFAM BLAST-PRODOM BLAST-DOMO

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
9	2278688CD1	919	T177 T325 Y326 S379 S427 S547 T548 S549 S632 T633 S667 T669 T721 T758 T759 S32 S33 T143 T325 Y326 S341 S342 S486 S522	N62 N484 N648	Membrane alanyl dipeptidase: PR00756: R185-F200, F235-V250, F313-L323, V349-T364, W368-W380  Zinc, aminopeptidase, metallopeptidase, neutral: DM00700 P164606 80-887: R53-Y842  Zinc protease: V349-Q357 Leucine zipper: L3-P23  Signal peptide: M1-S39 Peptidase family M1: L54-G441 Aminopeptidase, hydrolase, metalloprotease, zinc, N- glycoprotein, transmembrane, signal, anchor, membrane: PD001134: R53-S486  Zinc, aminopeptidase, metallopeptidase, neutral: DM00700 P37898 1-794: E52-G845  Pyroglutamyl peptidase: K6-L182 Pyrrolidone carboxyl peptidase: PR00140: T11-L31, S66-E85 (P<0.0041)  Peptidase, carboxylate, pyrrolidone, pyroglutamyl: DM03107 P42673 1-212: K6-G145  Carboxylesterase domain: E4-W62  Esterase, hydrolase, precursor, signal, glycoprotein, serine, carboxylesterase family: PD000169: K3-W62  Cholinesterase: DM00390 Q04791 355-538: K3-W62 Type B carboxylesterase: W15-N25	BLIMPS- PRINTS  BLAST-DOMO  MOTIFS MOTIFS HMMER HMME-PFAM BLAST- PRODOM  BLAST-DOMO  HMME-PFAM BLAST- PRODOM  BLAST-DOMO  BLIMPS- BLOCKS
10	4043361CD1	209	S118	N22		HMME-PFAM BLIMPS- PRINTS
11	3937958CD1	77	Y35 T47 S68			BLAST-DOMO  HMME-PFAM BLAST- PRODOM  BLAST-DOMO  BLIMPS- BLOCKS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
12	7257324CD1	414	S93 T94 T223 T258 T16 S26 T124 S182 S235 S300 S346 S396 Y118	N221 N233 N267	Serpins protein signatures BL00284: N71-T94, A173-I193, T200-M241, V306-F332, D387-P411 Serpins signature: G364-K414 Serpins, serine protease inhibitor, signal, precursor, glycoprotein, plasma, proteinase: PD000192: A44-P411 Serpins: DM00112 P01009 47-413: D54-N410 Signal peptide: M1-G19 Serpins (serine protease inhibitors): A45-P411	BLIMPS- BLOCKS ProfileScan BLAST- PRODOM BLAST-DOMO HMMER SPSCAN HMMER-PFAM
13	7472038CD1	397	S127 T166 S317 T381 S337 Y340 S16 S31 T90 T154 S252	N156 N166 N169 N178 N190 N195 N245 N298 N245 N298	Pepsin (A1) aspartic protease signature PR00792A: I84-V104, G230-T243, V278-L289, W369-D384 Aspartyl protease, hydrolase precursor, signal, zymogen, glycoprotein, multigene: P69-S307 Eukaryotic and viral aspartyl proteases: DM00126 Q03168 19-385: R23-A395 Aspartyl protease: V93-V104, V278-L289 Eukaryotic aspartyl protease: P69-A395 Eukaryotic and viral aspartic proteases BL00141: F91-S106, D184-S195, G235-G244, V278-L287, I370-A393	BLIMPS- PRINTS BLAST- PRODOM BLAST-DOMO MOTIFS HMMER-PFAM BLIMPS- BLOCKS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
14	7472041CD1	145	T76 S13 S19 S37 T83 S105	N42 N54 N57 N94 N98 N131 N132	Cysteine proteases, inhibitors: DM00182 P01035 1-110: G30-C134 Cysteine proteases inhibitor: R66-T89 Signal peptide: M1-G23 Cystatin domain: G30-S133 Cysteine proteases inhibitors signature: N53-S100	BLAST-DOMO BLIMPS- BLOCKS HMMER SPScan HMMER-PFAM ProfileScan



Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragments	Sequence Fragments	5' Position	3' Position
15	1714846CB1	4028	1349-1416, 1-199, 1903-3217	6831476H1 (SINTNOR01)	1	499
				6773219J1 (OVARDIR01)	650	1271
				6426758H1 (LUNGNON07)	998	1685
				1870084F6 (SKINBIT01)	1575	1995
				898127H1 (BRSTNOT05)	1964	2210
				6433334H1 (LUNGNON07)	1999	2596
				4442573H1 (SINTNOT22)	2572	2868
				6286315H2 (EPIPUNA01)	2586	3110
				1714846F6 (UCMCNOT02)	3058	3631
				257076T6 (HNT2RAT01)	3300	3988
				6487217H1 (MIXDUNB01)	3635	4028
				95364348	385	839
				70152356V1	1	569
				70161001V1	359	824
				70157441V1	686	1218
16	1856589CB1	1422	539-570, 324-395, 1-214, 756-933	60106256B2	976	1422
				548654H1 (BEPINOT01)	1	268
				2170381F6 (ENDCNOT03)	150	675
				1437060F1 (PANCNOT08)	476	1031
				70098221V1	774	1352
				1428845H1 (SINTBST01)	1170	1408
				3290066H1 (BONRFET01)	1318	1569
				2994130H1 (KIDNFET02)	1423	1715
				3601537H1 (DRGTNOT01)	1644	1850
				3702672H1 (PENCNOT07)	1760	1911
				754098R1 (BRAITUT02)	1	386
				70186361V1	143	847
				70186120V1	432	854
				3494839F6 (ADRETUT07)	1	685
				70005795D1	660	1266
17	2617672CB1	1911	1-619	2630625T6 (COLNTUT15)	708	1364
				605612H1 (BRSTTUT01)	1198	1385
18	2769104CB1	854	1-176			
19	4802789CB1	1386	1-23, 343-503			

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragments	Sequence Fragments	5' Position	3' Position
20	60116897CB1	3323	2502-2610, 1-735 1122-1879	3154611F6 (TLYMTXT02)	1	834
				60116918U1	740	1236
				2832568F6 (TLYMNOT03)	1119	1657
				2830930F7 (TLYMNOT03)	1610	2078
				6510679H1 (LUNGTUA01)	1877	2180
				2849992F6 (BRSTTUT13)	2135	2641
				3200003F6 (PENCNOT02)	2368	2862
				2849992F6 (BRSTTUT13)	2792	3323
				3201617F6 (PENCNOT02)	1219	1713
				824817R1 (PROSNOT06)	1	551
21	1866356	2123	1-1590	3257810H1 (PROSTUS08)	2004	2123
				5726464H1 (UTRSTUT05)	244	904
				3739625T6 (MENTNOT01)	1669	2075
				258590R6 (HNT2RAT01)	642	1073
				6157882H1 (MONOTXN05)	1821	2092
				6269726H1 (BRAIFEN03)	1046	1705
				4570803H1 (GBLADIT02)	1	249
				267175H1 (HNT2NOT01)	555	927
				1442881T6 (THYRNOT03)	2234	2893
				1388162H1 (CARGDIT02)	1368	1619
22	1872095	2893	584-1266, 1-56, 2839-2893	4662176H2 (BRSTTUT20)	1545	1809
				1344669H1 (PROSNOT11)	1714	1962
				SXBC01873V1	1898	2461
				449756R6 (TLYMNOT02)	219	734
				449756T6 (TLYMNOT02)	797	1459
				SXBC00314V1	1950	2515

Table 4 (cont.)

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragments	Sequence Fragments	5' Position	3' Position
23	2278688	4170	1-245, 3069-3624, 1149-1809	2254713H1 (OVRTUT01) 097483R1 (PITUNOR01) 3271744H1 (BRAINOT20) 4422961H1 (BRAPDIT01) 1378162H1 (LUNGNOT10) 3076825H1 (BONEUNT01) 3556490H1 (LUNGNOT31) 1368447H1 (SCORNON02) 4662177H2 (BRSTTUT20) 3853790H1 (BRAITUT12) 1877059H1 (LEUKNOT03) 1349282T1 (LATRTUT02) 4289627F6 (BRABDIR01) 1289505T1 (BRAINOT11) 2373989F6 (ISLTNOT01) 097483F1 (PITUNOR01) 2698679H1 (UTRSNOT12) 2110561H1 (BRAITUT03) 3011419H1 (MUSCNOT07) 1394210H1 (THYRNOT03) 4880281H1 (UTRMTT01) 4043361F6 (LUNGNOT35) 6777288J1 (OVARDIR01) 6121924H1 (BRAHNON05) 7032724H1 (BRAXTDRI2) 4692968T6 (BRAENOT02) 1871340F6 (SKINBIT01) 3429631T6 (SKINNOT04) 7257324H1 (SKIRTDC01) GNN.g6436155_002.edit GNN.g5830433_004.edit	453 2611 1433 1258 1110 2127 759 3998 1 2411 2062 3814 72 3530 1570 2933 1817 669 2369 1001 524 1 436 1022 1 636 1256 416 1 1 1 1	710 3220 1667 1500 1309 2391 1064 4170 271 2705 2330 4157 578 4149 2094 3639 2134 950 2623 1296 767 593 1216 1538 480 1265 1497 1476 474 1194 438
24	4043361	767	1-66			
25	3937958	1538	385-506, 1-78, 1293-1538			
26	7257324	1497	651-770, 67-206			
27	7472038	1194	1-29, 788-1194			
28	7472041	438	1-27			

Table 5

Polynucleotide SEQ ID NO:	Incyte Project ID	Representative Library
15	17114846CB1	LUNGNON07
16	1856589CB1	PROSNOT18
17	2617672CB1	PANCNOT08
18	2769104CB1	COLANOT02
19	4802789CB1	ADRETUT07
20	60116897CB1	TYMNOT03
21	1866356CB1	HNT2RAT01
22	1872095CB1	THYRNOT03
23	2278688CB1	LATRTUT02
24	4043361CB1	LUNGNOT35
25	3937958CB1	KIDNNOT05
26	7257324CB1	SKINNNOT04

Table 6

Library	Vector	Library Description
LUNGNON07	pINCY	This normalized lung tissue library was constructed from RNA isolated from a lung tissue library. The library was normalized in two rounds using conditions adapted from Soares et al. (1994) Proc. Natl. Acad. Sci. USA 91:9228-9232 and Bonaldo et al. (1996) Genome Res. 6:791, except that a significantly longer (48 hours/round) reannealing hybridization was used.
PROSNOT18	pINCY	Library was constructed using RNA isolated from diseased prostate tissue removed from a 58-year-old Caucasian male during a radical cystectomy, radical prostatectomy, and gastrectomy. Pathology indicated adenofibromatous hyperplasia; this tissue was associated with a grade 3 transitional cell carcinoma. Patient history included angina and emphysema. Family history included acute myocardial infarction, atherosclerotic coronary artery disease, and type II diabetes.
PANCNOT08	pINCY	Library was constructed using RNA isolated from pancreatic tissue removed from a 65-year-old Caucasian female during radical subtotal pancreatectomy. Pathology for the associated tumor tissue indicated an invasive grade 2 adenocarcinoma. Patient history included type II diabetes, osteoarthritis, cardiovascular disease, benign neoplasm in the large bowel, and a cataract. Previous surgeries included a total splenectomy, cholecystectomy, and abdominal hysterectomy. Family history included cardiovascular disease, type II diabetes, and stomach cancer.
COLANOT02	pINCY	Library was constructed using RNA isolated from diseased ascending colon tissue removed from a 25-year-old Caucasian female during a multiple segmental resection of the large bowel. Pathology indicated moderately to severely active chronic ulcerative colitis, involving the entire colectomy specimen and sparing 2 cm of the attached ileum. Grossly, the specimen showed continuous involvement from the rectum proximally; marked mucosal atrophy and no skip areas were identified. Microscopically, the specimen showed dense, predominantly mucosal inflammation and crypt abscesses. Patient history included benign large bowel neoplasm. Previous surgeries included a polypectomy.
ADRETUT07	pINCY	Library was constructed using RNA isolated from adrenal tumor tissue removed from a 43-year-old Caucasian female during a unilateral adrenalectomy. Pathology indicated pheochromocytoma.

Table 6 (cont.)

Library	Vector	Library Description
TYMNOT03	pINCY	Library was constructed using RNA isolated from nonactivated Th1 cells. These cells were differentiated from umbilical cord CD4 T cells with IL-12 and B7-transfected COS cells.
HNT2RAT01	PBLUESCRIPT	Library was constructed at Stratagene (STR937231), using RNA isolated from the hNT2 cell line (derived from a human teratocarcinoma that exhibited properties characteristic of a committed neuronal precursor). Cells were treated with retinoic acid for 24 hours.
LATRTUT02	pINCY	Library was constructed using RNA isolated from a myxoma removed from the left atrium of a 43-year-old Caucasian male during annuloplasty. Pathology indicated atrial myxoma. Patient history included pulmonary insufficiency, acute myocardial infarction, atherosclerotic coronary artery disease, hyperlipidemia, and tobacco use. Family history included benign hypertension, acute myocardial infarction, atherosclerotic coronary artery disease, and type II diabetes.
LUNGNOT35	pINCY	Library was constructed using RNA isolated from lung tissue removed from a 62-year-old Caucasian female. Pathology for the associated tumor tissue indicated a grade 1 spindle cell carcinoma forming a nodule. Patient history included depression, thrombophlebitis, and hyperlipidemia. Family history included cerebrovascular disease, atherosclerotic coronary artery disease, breast cancer, colon cancer, type II diabetes, and malignant skin melanoma.
THYRNOT03	pINCY	Library was constructed using RNA isolated from thyroid tissue removed from the left thyroid of a 28-year-old Caucasian female during a complete thyroidectomy. Pathology indicated a small nodule of adenomatous hyperplasia present in the left thyroid. Pathology for the associated tumor tissue indicated dominant follicular adenoma, forming a well-encapsulated mass in the left thyroid.
KIDNNOT05	PSPORT1	Library was constructed using RNA isolated from the kidney tissue of a 2-day-old Hispanic female, who died from cerebral anoxia. Family history included congenital heart disease.
SKINNOT04	pINCY	Library was constructed using RNA isolated from breast skin tissue removed from a 70-year-old Caucasian female during a breast biopsy and resection.

Table 7

Program	Description	Reference	Parameter Threshold
ABIFACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less Full Length sequences: Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 Assembled ESTs: fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less Full Length sequences: fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1998) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value= 1.0E-3 or less Signal peptide hits: Score= 0 or greater

Table 7 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribkov, M. et al. (1988) CABIOS 4:61-66; Gribkov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score>GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
TMAP	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
TMHMMER	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	



What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
  - 5 a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-14,
  - b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-14,
  - c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14, and
  - 10 d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.
2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-14.
- 15 3. An isolated polynucleotide encoding a polypeptide of claim 1.
4. An isolated polynucleotide encoding a polypeptide of claim 2.
- 20 5. An isolated polynucleotide of claim 4 selected from the group consisting of SEQ ID NO:15-28.
6. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.
- 25 7. A cell transformed with a recombinant polynucleotide of claim 6.
8. A transgenic organism comprising a recombinant polynucleotide of claim 6.
- 30 9. A method for producing a polypeptide of claim 1, the method comprising:
  - a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and
  - 35 b) recovering the polypeptide so expressed.

10. An isolated antibody which specifically binds to a polypeptide of claim 1.

11. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

- 5       a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28,  
      b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:15-28,  
      c) a polynucleotide sequence complementary to a),  
      d) a polynucleotide sequence complementary to b), and  
10       e) an RNA equivalent of a)-d).

12. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a polynucleotide of claim 11.

15       13. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

- a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization  
20       complex is formed between said probe and said target polynucleotide or fragments thereof, and  
      b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

25       14. A method of claim 13, wherein the probe comprises at least 60 contiguous nucleotides.

      15. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

- a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and  
30       b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

16. A composition comprising an effective amount of a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

35

17. A composition of claim 16, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-14.

18. A method for treating a disease or condition associated with decreased expression of functional PRTS, comprising administering to a patient in need of such treatment the composition of claim 16.

19. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting agonist activity in the sample.

20. A composition comprising an agonist compound identified by a method of claim 19 and a pharmaceutically acceptable excipient.

21. A method for treating a disease or condition associated with decreased expression of functional PRTS, comprising administering to a patient in need of such treatment a composition of claim 20.

22. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

23. A composition comprising an antagonist compound identified by a method of claim 22 and a pharmaceutically acceptable excipient.

24. A method for treating a disease or condition associated with overexpression of functional PRTS, comprising administering to a patient in need of such treatment a composition of claim 23.

25. A method of screening for a compound that specifically binds to the polypeptide of claim 1, said method comprising the steps of:

- a) combining the polypeptide of claim 1 with at least one test compound under suitable conditions, and
- b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a

compound that specifically binds to the polypeptide of claim 1.

26. A method of screening for a compound that modulates the activity of the polypeptide of claim 1, said method comprising:

- 5           a) combining the polypeptide of claim 1 with at least one test compound under conditions permissive for the activity of the polypeptide of claim 1,
- b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound, and
- c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change in
- 10          the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide of claim 1.

27. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method

15          comprising:

- a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,
- b) detecting altered expression of the target polynucleotide, and
- c) comparing the expression of the target polynucleotide in the presence of varying amounts of
- 20          the compound and in the absence of the compound.

28. A method for assessing toxicity of a test compound, said method comprising:

- a) treating a biological sample containing nucleic acids with the test compound;
- b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at
- 25          least 20 contiguous nucleotides of a polynucleotide of claim 11 under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 11 or fragment thereof;
- c) quantifying the amount of hybridization complex; and
- 30          d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

<110> INCYTE GENOMICS, INC.  
 YANG, Junming  
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 NGUYEN, Danniell B.  
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Asn	Asp	Thr	Gln	Ile	Leu	Gln	Gln	Ala	Leu	Lys	Asp	Ser	Asn	Gly
				35					40					45
Asn	Leu	Glu	Leu	Ala	Val	Ala	Phe	Leu	Thr	Ala	Lys	Asn	Ala	Lys
				50					55					60
Thr	Pro	Gln	Gln	Glu	Glu	Thr	Thr	Tyr	Tyr	Gln	Thr	Ala	Leu	Pro
				65					70					75
Gly	Asn	Asp	Arg	Tyr	Ile	Ser	Val	Gly	Ser	Gln	Ala	Asp	Thr	Asn
				80					85					90
Val	Ile	Asp	Leu	Thr	Gly	Asp	Asp	Lys	Asp	Asp	Leu	Gln	Arg	Ala
				95					100					105
Ile	Ala	Leu	Ser	Leu	Ala	Glu	Ser	Asn	Arg	Ala	Phe	Arg	Glu	Thr
				110					115					120
Gly	Ile	Thr	Asp	Glu	Glu	Gln	Ala	Ile	Ser	Arg	Val	Leu	Glu	Ala
				125					130					135
Ser	Ile	Ala	Glu	Asn	Lys	Ala	Cys	Leu	Lys	Arg	Thr	Pro	Thr	Glu
				140					145					150
Val	Trp	Arg	Asp	Ser	Arg	Asn	Pro	Tyr	Asp	Arg	Lys	Arg	Gln	Asp
				155					160					165
Lys	Ala	Pro	Val	Gly	Leu	Lys	Asn	Val	Gly	Asn	Thr	Cys	Trp	Phe
				170					175					180
Ser	Ala	Val	Ile	Gln	Ser	Leu	Phe	Asn	Leu	Leu	Glu	Phe	Arg	Arg
				185					190					195
Leu	Val	Leu	Asn	Tyr	Lys	Pro	Pro	Ser	Asn	Ala	Gln	Asp	Leu	Pro
				200					205					210
Arg	Asn	Gln	Lys	Glu	His	Arg	Asn	Leu	Pro	Phe	Met	Arg	Glu	Leu
				215					220					225
Arg	Tyr	Leu	Phe	Ala	Leu	Leu	Val	Gly	Thr	Lys	Arg	Lys	Tyr	Val

	230		235		240
Asp Pro Ser Arg	Ala Val Glu Ile Leu	Lys Asp Ala Phe Lys	Ser		
	245		250		255
Asn Asp Ser Gln	Gln Gln Asp Val Ser	Glu Phe Thr His Lys	Leu		
	260		265		270
Leu Asp Trp Leu	Glu Asp Ala Phe Gln	Met Lys Ala Glu Glu	Glu		
	275		280		285
Thr Asp Glu Glu	Lys Pro Lys Asn Pro	Met Val Glu Leu Phe	Tyr		
	290		295		300
Gly Arg Phe Leu	Ala Val Gly Val Leu	Glu Gly Lys Lys Phe	Glu		
	305		310		315
Asn Thr Glu Met	Phe Gly Gln Tyr Pro	Leu Gln Val Asn Gly	Phe		
	320		325		330
Lys Asp Leu His	Glu Cys Leu Glu Ala	Ala Met Ile Glu Gly	Glu		
	335		340		345
Ile Glu Ser Leu	His Ser Glu Asn Ser	Gly Lys Ser Gly Gln	Glu		
	350		355		360
His Trp Phe Thr	Glu Leu Pro Pro Val	Leu Thr Phe Glu Leu	Ser		
	365		370		375
Arg Phe Glu Phe	Asn Gln Ala Leu Gly	Arg Pro Glu Lys Ile	His		
	380		385		390
Asn Lys Leu Glu	Phe Pro Gln Val Leu	Tyr Leu Asp Arg Tyr	Met		
	395		400		405
His Arg Asn Arg	Glu Ile Thr Arg Ile	Lys Arg Glu Glu Ile	Lys		
	410		415		420
Arg Leu Lys Asp	Tyr Leu Thr Val Leu	Gln Gln Arg Leu Glu	Arg		
	425		430		435
Tyr Leu Ser Tyr	Gly Ser Gly Pro Lys	Arg Phe Pro Leu Val	Asp		
	440		445		450
Val Leu Gln Tyr	Ala Leu Glu Phe Ala	Ser Ser Lys Pro Val	Cys		
	455		460		465
Thr Ser Pro Val	Asp Asp Ile Asp Ala	Ser Ser Pro Pro Ser	Gly		
	470		475		480
Ser Ile Pro Ser	Gln Thr Leu Pro Ser	Thr Thr Glu Gln Gln	Gly		
	485		490		495
Ala Leu Ser Ser	Glu Leu Pro Ser Thr	Ser Pro Ser Ser Val	Ala		
	500		505		510
Ala Ile Ser Ser	Arg Ser Val Ile His	Lys Pro Phe Thr Gln	Ser		
	515		520		525
Arg Ile Pro Pro	Asp Leu Pro Met His	Pro Ala Pro Arg His	Ile		
	530		535		540
Thr Glu Glu Glu	Glu Ser Val Leu Glu	Ser Cys Leu His Arg	Trp		
	545		550		555
Arg Thr Glu Ile	Glu Asn Asp Thr Arg	Asp Leu Gln Glu Ser	Ile		
	560		565		570
Ser Arg Ile His	Arg Thr Ile Glu Leu	Met Tyr Ser Asp Lys	Ser		
	575		580		585
Met Ile Gln Val	Pro Tyr Arg Leu His	Ala Val Leu Val His	Glu		
	590		595		600
Gly Gln Ala Asn	Ala Gly His Tyr Trp	Ala Tyr Ile Phe Asp	His		
	605		610		615
Arg Glu Ser Arg	Trp Met Lys Tyr Asn	Asp Ile Ala Val Thr	Lys		
	620		625		630
Ser Ser Trp Glu	Glu Leu Val Arg Asp	Ser Phe Gly Gly Tyr	Arg		
	635		640		645
Asn Ala Ser Ala	Tyr Cys Leu Met Tyr	Ile Asn Asp Lys Ala	Gln		
	650		655		660
Phe Leu Ile Gln	Glu Glu Phe Asn Lys	Glu Thr Gly Gln Pro	Leu		
	665		670		675
Val Gly Ile Glu	Thr Leu Pro Pro Asp	Leu Arg Asp Phe Val	Glu		
	680		685		690
Glu Asp Asn Gln	Arg Phe Glu Lys Glu	Leu Glu Glu Trp Asp	Ala		
	695		700		705
Gln Leu Ala Gln	Lys Ala Leu Gln Glu	Lys Leu Leu Ala Ser	Gln		
	710		715		720
Lys Leu Arg Glu	Ser Glu Thr Ser Val	Thr Thr Ala Gln Ala	Ala		
	725		730		735

Gly	Asp	Pro	Glu	Tyr	Leu	Glu	Gln	Pro	Ser	Arg	Ser	Asp	Phe	Ser	
				740					745					750	
Lys	His	Leu	Lys	Glu	Glu	Thr	Ile	Gln	Ile	Ile	Thr	Lys	Ala	Ser	
				755					760					765	
His	Glu	His	Glu	Asp	Lys	Ser	Pro	Glu	Thr	Val	Leu	Gln	Ser	Ala	
				770					775					780	
Ile	Lys	Leu	Glu	Tyr	Ala	Arg	Leu	Val	Lys	Leu	Ala	Gln	Glu	Asp	
				785					790					795	
Thr	Pro	Pro	Glu	Thr	Asp	Tyr	Arg	Leu	His	His	Val	Val	Val	Tyr	
				800					805					810	
Phe	Ile	Gln	Asn	Gln	Ala	Pro	Lys	Lys	Ile	Ile	Glu	Lys	Thr	Leu	
				815					820					825	
Leu	Glu	Gln	Phe	Gly	Asp	Arg	Asn	Leu	Ser	Phe	Asp	Glu	Arg	Cys	
				830					835					840	
His	Asn	Ile	Met	Lys	Val	Ala	Gln	Ala	Lys	Leu	Glu	Met	Ile	Lys	
				845					850					855	
Pro	Glu	Glu	Val	Asn	Leu	Glu	Glu	Tyr	Glu	Glu	Trp	His	Gln	Asp	
				860					865					870	
Tyr	Arg	Lys	Phe	Arg	Glu	Thr	Thr	Met	Tyr	Leu	Ile	Ile	Gly	Leu	
				875					880					885	
Glu	Asn	Phe	Gln	Arg	Glu	Ser	Tyr	Ile	Asp	Ser	Leu	Leu	Phe	Leu	
				890					895					900	
Ile	Cys	Ala	Tyr	Gln	Asn	Asn	Lys	Glu	Leu	Leu	Ser	Lys	Gly	Leu	
				905					910					915	
Tyr	Arg	Gly	His	Asp	Glu	Glu	Leu	Ile	Ser	His	Tyr	Arg	Arg	Glu	
				920					925					930	
Cys	Leu	Leu	Lys	Leu	Asn	Glu	Gln	Ala	Ala	Glu	Leu	Phe	Glu	Ser	
				935					940					945	
Gly	Glu	Asp	Arg	Glu	Val	Asn	Asn	Gly	Leu	Ile	Ile	Met	Asn	Glu	
				950					955					960	
Phe	Ile	Val	Pro	Phe	Leu	Pro	Leu	Leu	Leu	Val	Asp	Glu	Met	Glu	
				965					970					975	
Glu	Lys	Asp	Ile	Leu	Ala	Val	Glu	Asp	Met	Arg	Asn	Arg	Trp	Cys	
				980					985					990	
Ser	Tyr	Leu	Gly	Gln	Glu	Met	Glu	Pro	His	Leu	Gln	Glu	Lys	Leu	
				995					1000					1005	
Thr	Asp	Phe	Leu	Pro	Lys	Leu	Leu	Asp	Cys	Ser	Met	Glu	Ile	Lys	
				1010					1015					1020	
Ser	Phe	His	Glu	Pro	Pro	Lys	Leu	Pro	Ser	Tyr	Ser	Thr	His	Glu	
				1025					1030					1035	
Leu	Cys	Glu	Arg	Phe	Ala	Arg	Ile	Met	Leu	Ser	Leu	Ser	Arg	Thr	
				1040					1045					1050	
Pro	Ala	Asp	Gly	Arg											
				1055											

&lt;210&gt; 2

&lt;211&gt; 358

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1856589CD1

&lt;400&gt; 2

Met	Gly	Ala	Ala	Thr	Cys	Arg	Gly	Ser	Arg	Ile	Pro	Ser	Gly	Pro	
1				5					10					15	
Pro	Val	Gln	Gly	Glu	Arg	Ser	Ala	Pro	Arg	Phe	Gly	Val	Thr	Ser	
				20					25					30	
Leu	Ser	Leu	Trp	Pro	Ala	Asp	Phe	Lys	Asp	Asn	Trp	Arg	Ile	Ala	
				35					40					45	
Gly	Ser	Arg	Gln	Glu	Val	Ala	Leu	Ala	Gly	Glu	Pro	Ala	Asp	Gln	
				50					55					60	
Gln	Gln	Thr	His	Leu	Arg	Arg	Leu	Pro	Tyr	Arg	Gln	Thr	Leu	Gly	
				65					70					75	
Tyr	Lys	Glu	Asp	Thr	Thr	Asn	Pro	Val	Cys	Gly	Glu	Pro	Trp	Trp	
				80					85					90	

Ser	Glu	Asp	Leu	Glu	Met	Thr	Arg	His	Trp	Pro	Trp	Glu	Val	Ser	
				95					100					105	
Leu	Arg	Met	Glu	Asn	Glu	His	Val	Cys	Gly	Gly	Ala	Leu	Ile	Asp	
				110					115					120	
Pro	Ser	Trp	Val	Val	Thr	Ala	Ala	His	Cys	Ser	Gln	Gly	Thr	Lys	
				125					130					135	
Glu	Tyr	Ser	Val	Val	Leu	Gly	Thr	Ser	Lys	Leu	Gln	Pro	Met	Asn	
				140					145					150	
Phe	Ser	Arg	Ala	Leu	Trp	Val	Pro	Val	Arg	Asp	Ile	Ile	Met	His	
				155					160					165	
Pro	Lys	Tyr	Trp	Gly	Arg	Ala	Phe	Ile	Met	Gly	Asp	Val	Ala	Leu	
				170					175					180	
Val	His	Leu	Gln	Thr	Pro	Val	Thr	Phe	Ser	Glu	Tyr	Val	Gln	Pro	
				185					190					195	
Ile	Cys	Leu	Pro	Glu	Pro	Asn	Phe	Asn	Leu	Lys	Val	Gly	Thr	Gln	
				200					205					210	
Cys	Trp	Val	Thr	Gly	Trp	Ser	Gln	Val	Lys	Gln	Arg	Phe	Ser	Gly	
				215					220					225	
Ser	Thr	Ala	Asn	Ser	Met	Leu	Thr	Pro	Glu	Leu	Gln	Glu	Ala	Glu	
				230					235					240	
Val	Phe	Ile	Met	Asp	Asn	Lys	Arg	Cys	Asp	Arg	His	Tyr	Lys	Lys	
				245					250					255	
Ser	Phe	Phe	Pro	Leu	Val	Val	Pro	Leu	Val	Leu	Gly	Asp	Met	Ile	
				260					265					270	
Cys	Ala	Thr	Asn	Tyr	Gly	Glu	Asn	Leu	Cys	Tyr	Gly	Asp	Ser	Gly	
				275					280					285	
Gly	Pro	Leu	Ala	Cys	Glu	Val	Glu	Gly	Arg	Trp	Ile	Leu	Ala	Gly	
				290					295					300	
Val	Leu	Ser	Trp	Glu	Lys	Ala	Cys	Val	Lys	Ala	Gln	Asn	Pro	Gly	
				305					310					315	
Val	Tyr	Thr	Arg	Val	Thr	Lys	Tyr	Thr	Lys	Trp	Ile	Lys	Lys	Gln	
				320					325					330	
Met	Ser	Asn	Gly	Ala	Phe	Ser	Gly	Pro	Cys	Ala	Ser	Ala	Cys	Leu	
				335					340					345	
Leu	Phe	Leu	Cys	Trp	Pro	Leu	Gln	Pro	Gln	Met	Gly	Ser			
				350					355						

&lt;210&gt; 3

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2617672CD1

&lt;400&gt; 3

Met	Trp	Arg	Cys	Pro	Leu	Gly	Leu	Leu	Leu	Leu	Leu	Pro	Leu	Ala	
1				5					10					15	
Gly	His	Leu	Ala	Leu	Gly	Ala	Gln	Gln	Gly	Arg	Gly	Arg	Arg	Glu	
				20					25					30	
Leu	Ala	Pro	Gly	Leu	His	Leu	Arg	Gly	Ile	Arg	Asp	Ala	Gly	Gly	
				35					40					45	
Arg	Tyr	Cys	Gln	Glu	Gln	Asp	Leu	Cys	Cys	Arg	Gly	Arg	Ala	Asp	
				50					55					60	
Asp	Cys	Ala	Leu	Pro	Tyr	Leu	Gly	Ala	Ile	Cys	Tyr	Cys	Asp	Leu	
				65					70					75	
Phe	Cys	Asn	Arg	Thr	Val	Ser	Asp	Cys	Cys	Pro	Asp	Phe	Trp	Asp	
				80					85					90	
Phe	Cys	Leu	Gly	Val	Pro	Pro	Pro	Phe	Pro	Pro	Ile	Gln	Gly	Cys	
				95					100					105	
Met	His	Gly	Gly	Arg	Ile	Tyr	Pro	Val	Leu	Gly	Thr	Tyr	Trp	Asp	
				110					115					120	
Asn	Cys	Asn	Arg	Cys	Thr	Cys	Gln	Glu	Asn	Arg	Gln	Trp	Gln	Cys	
				125					130					135	
Asp	Gln	Glu	Pro	Cys	Leu	Val	Asp	Pro	Asp	Met	Ile	Lys	Ala	Ile	
				140					145					150	



Asn	Gln	Gly	Asn	Tyr	Gly	Trp	Gln	Ala	Gly	Asn	His	Ser	Ala	Phe
				155					160					165
Trp	Gly	Met	Thr	Leu	Asp	Glu	Gly	Ile	Arg	Tyr	Arg	Leu	Gly	Thr
				170					175					180
Ile	Arg	Pro	Ser	Ser	Ser	Val	Met	Asn	Met	His	Glu	Ile	Tyr	Thr
				185					190					195
Val	Leu	Asn	Pro	Gly	Glu	Val	Leu	Pro	Thr	Ala	Phe	Glu	Ala	Ser
				200					205					210
Glu	Lys	Trp	Pro	Asn	Leu	Ile	His	Glu	Pro	Leu	Asp	Gln	Gly	Asn
				215					220					225
Cys	Ala	Gly	Ser	Trp	Ala	Phe	Ser	Thr	Ala	Ala	Val	Ala	Ser	Asp
				230					235					240
Arg	Val	Ser	Ile	His	Ser	Leu	Gly	His	Met	Thr	Pro	Val	Leu	Ser
				245					250					255
Pro	Gln	Asn	Leu	Leu	Ser	Cys	Asp	Thr	His	Gln	Gln	Gln	Gly	Cys
				260					265					270
Arg	Gly	Gly	Arg	Leu	Asp	Gly	Ala	Trp	Trp	Phe	Leu	Arg	Arg	Arg
				275					280					285
Gly	Val	Val	Ser	Asp	His	Cys	Tyr	Pro	Phe	Ser	Gly	Arg	Glu	Arg
				290					295					300
Asp	Glu	Ala	Gly	Pro	Ala	Pro	Pro	Cys	Met	Met	His	Ser	Arg	Ala
				305					310					315
Met	Gly	Arg	Gly	Lys	Arg	Gln	Ala	Thr	Ala	His	Cys	Pro	Asn	Ser
				320					325					330
Tyr	Val	Asn	Asn	Asn	Asp	Ile	Tyr	Gln	Val	Thr	Pro	Val	Tyr	Arg
				335					340					345
Leu	Gly	Ser	Asn	Asp	Lys	Glu	Ile	Met	Lys	Glu	Leu	Met	Glu	Asn
				350					355					360
Gly	Pro	Val	Gln	Ala	Leu	Met	Glu	Val	His	Glu	Asp	Phe	Phe	Leu
				365					370					375
Tyr	Lys	Gly	Gly	Ile	Tyr	Ser	His	Thr	Pro	Val	Ser	Leu	Gly	Arg
				380					385					390
Pro	Glu	Arg	Tyr	Arg	Arg	His	Gly	Thr	His	Ser	Val	Lys	Ile	Thr
				395					400					405
Gly	Trp	Gly	Glu	Glu	Thr	Leu	Pro	Asp	Gly	Arg	Thr	Leu	Lys	Tyr
				410					415					420
Trp	Thr	Ala	Ala	Asn	Ser	Trp	Gly	Pro	Ala	Trp	Gly	Glu	Arg	Gly
				425					430					435
His	Phe	Arg	Ile	Val	Arg	Gly	Val	Asn	Glu	Cys	Asp	Ile	Glu	Ser
				440					445					450
Phe	Val	Leu	Gly	Val	Trp	Gly	Arg	Val	Gly	Met	Glu	Asp	Met	Gly
				455					460					465

His His

&lt;210&gt; 4

&lt;211&gt; 187

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2769104CD1

&lt;400&gt; 4

Met	Pro	Gly	Pro	Arg	Val	Trp	Gly	Lys	Tyr	Leu	Trp	Arg	Ser	Pro
1				5					10					15
His	Ser	Lys	Gly	Cys	Pro	Gly	Ala	Met	Trp	Trp	Leu	Leu	Leu	Trp
				20					25					30
Gly	Val	Leu	Gln	Ala	Cys	Pro	Thr	Arg	Gly	Ser	Val	Leu	Leu	Ala
				35					40					45
Gln	Glu	Leu	Pro	Gln	Gln	Leu	Thr	Ser	Pro	Gly	Tyr	Pro	Glu	Pro
				50					55					60
Tyr	Gly	Lys	Gly	Gln	Glu	Ser	Ser	Thr	Asp	Ile	Lys	Ala	Pro	Glu
				65					70					75
Gly	Phe	Ala	Val	Arg	Leu	Val	Phe	Gln	Asp	Phe	Asp	Leu	Glu	Pro
				80					85					90

Ser	Gln	Asp	Cys	Ala	Gly	Asp	Ser	Val	Thr	Ile	Ser	Phe	Val	Gly	
				95					100					105	
Ser	Asp	Pro	Ser	Gln	Phe	Cys	Gly	Gln	Gln	Gly	Ser	Pro	Leu	Gly	
				110					115					120	
Arg	Pro	Pro	Gly	Gln	Arg	Glu	Phe	Val	Ser	Ser	Gly	Arg	Ser	Leu	
				125					130					135	
Arg	Leu	Thr	Phe	Arg	Thr	Gln	Pro	Ser	Ser	Glu	Asn	Lys	Thr	Ala	
				140					145					150	
His	Leu	His	Lys	Gly	Phe	Leu	Ala	Leu	Tyr	Gln	Thr	Val	Gly	Glu	
				155					160					165	
Cys	Pro	Ser	Trp	Gly	Cys	Arg	Glu	Gly	Ala	Ser	Val	Pro	Ser	His	
				170					175					180	
Asp	Pro	Gly	Ile	Phe	Lys	Pro									
				185											

<210> 5  
 <211> 289  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 4802789CD1

<400> 5

Met	Arg	Val	Lys	Asp	Pro	Thr	Lys	Ala	Leu	Pro	Glu	Lys	Ala	Lys	
1				5					10					15	
Arg	Ser	Lys	Arg	Pro	Thr	Val	Pro	His	Asp	Glu	Asp	Ser	Ser	Asp	
				20					25					30	
Asp	Ile	Ala	Val	Gly	Leu	Thr	Cys	Gln	His	Val	Ser	His	Ala	Ile	
				35					40					45	
Ser	Val	Asn	His	Val	Lys	Arg	Ala	Ile	Ala	Glu	Asn	Leu	Trp	Ser	
				50					55					60	
Val	Cys	Ser	Glu	Cys	Leu	Lys	Glu	Arg	Arg	Phe	Tyr	Asp	Gly	Gln	
				65					70					75	
Leu	Val	Leu	Thr	Ser	Asp	Ile	Trp	Leu	Cys	Leu	Lys	Cys	Gly	Phe	
				80					85					90	
Gln	Gly	Cys	Gly	Lys	Asn	Ser	Glu	Ser	Gln	His	Ser	Leu	Lys	His	
				95					100					105	
Phe	Lys	Ser	Ser	Arg	Thr	Glu	Pro	His	Cys	Ile	Ile	Ile	Asn	Leu	
				110					115					120	
Ser	Thr	Trp	Ile	Ile	Trp	Cys	Tyr	Glu	Cys	Asp	Glu	Lys	Leu	Ser	
				125					130					135	
Thr	His	Cys	Asn	Lys	Lys	Val	Leu	Ala	Gln	Ile	Val	Asp	Phe	Leu	
				140					145					150	
Gln	Lys	His	Ala	Ser	Lys	Thr	Gln	Thr	Ser	Ala	Phe	Ser	Arg	Ile	
				155					160					165	
Met	Lys	Leu	Cys	Glu	Glu	Lys	Cys	Glu	Thr	Asp	Glu	Ile	Gln	Lys	
				170					175					180	
Gly	Gly	Lys	Cys	Arg	Asn	Leu	Ser	Val	Arg	Gly	Ile	Thr	Asn	Leu	
				185					190					195	
Gly	Asn	Thr	Cys	Phe	Phe	Asn	Ala	Val	Met	Gln	Asn	Leu	Ala	Gln	
				200					205					210	
Thr	Tyr	Thr	Leu	Thr	Asp	Leu	Met	Asn	Glu	Ile	Lys	Glu	Ser	Ser	
				215					220					225	
Thr	Lys	Leu	Lys	Ile	Phe	Pro	Ser	Ser	Asp	Ser	Gln	Leu	Asp	Pro	
				230					235					240	
Leu	Val	Val	Glu	Leu	Ser	Arg	Pro	Gly	Pro	Leu	Thr	Ser	Ala	Leu	
				245					250					255	
Phe	Leu	Phe	Leu	His	Ser	Met	Lys	Glu	Thr	Glu	Lys	Gly	Pro	Leu	
				260					265					270	
Ser	Pro	Lys	Val	Leu	Phe	Asn	Gln	Leu	Cys	Gln	Lys	Trp	Val	His	
				275					280					285	
Leu	His	Leu	Ile												

<210> 6

<211> 960  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 60116897CD1

<400> 6

Met	Phe	His	Ser	Ser	Ala	Met	Val	Asn	Ser	His	Arg	Lys	Pro	Met	1	5	10	15
Phe	Asn	Ile	His	Arg	Gly	Phe	Tyr	Cys	Leu	Thr	Ala	Ile	Leu	Pro	20	25	30	35
Gln	Ile	Cys	Ile	Cys	Ser	Gln	Phe	Ser	Val	Pro	Ser	Ser	Tyr	His	40	45	50	55
Phe	Thr	Glu	Asp	Pro	Gly	Ala	Phe	Pro	Val	Ala	Thr	Asn	Gly	Glu	60	65	70	75
Arg	Phe	Pro	Trp	Gln	Glu	Leu	Arg	Leu	Pro	Ser	Val	Val	Ile	Pro	80	85	90	95
Leu	His	Tyr	Asp	Leu	Phe	Val	His	Pro	Asn	Leu	Thr	Ser	Leu	Asp	100	105	110	115
Phe	Val	Ala	Ser	Glu	Lys	Ile	Glu	Val	Leu	Val	Ser	Asn	Ala	Thr	120	125	130	135
Gln	Phe	Ile	Ile	Leu	His	Ser	Lys	Asp	Leu	Glu	Ile	Thr	Asn	Ala	140	145	150	155
Thr	Leu	Gln	Ser	Glu	Glu	Asp	Ser	Arg	Tyr	Met	Lys	Pro	Gly	Lys	160	165	170	175
Glu	Leu	Lys	Val	Leu	Ser	Tyr	Pro	Ala	His	Glu	Gln	Ile	Ala	Leu	180	185	190	195
Leu	Val	Pro	Glu	Lys	Leu	Thr	Pro	His	Leu	Lys	Tyr	Tyr	Val	Ala	200	205	210	215
Met	Asp	Phe	Gln	Ala	Lys	Leu	Gly	Asp	Gly	Phe	Glu	Gly	Phe	Tyr	220	225	230	235
Lys	Ser	Thr	Tyr	Arg	Thr	Leu	Gly	Gly	Glu	Thr	Arg	Ile	Leu	Ala	240	245	250	255
Val	Thr	Asp	Phe	Glu	Pro	Thr	Gln	Ala	Arg	Met	Ala	Phe	Pro	Cys	260	265	270	275
Phe	Asp	Glu	Pro	Leu	Phe	Lys	Ala	Asn	Phe	Ser	Ile	Lys	Ile	Arg	280	285	290	295
Arg	Glu	Ser	Arg	His	Ile	Ala	Leu	Ser	Asn	Met	Pro	Lys	Val	Lys	300	305	310	315
Thr	Ile	Glu	Leu	Gly	Gly	Leu	Leu	Ser	Glu	Asp	His	Phe	Glu	Thr	320	325	330	335
Thr	Val	Lys	Met	Ser	Thr	Tyr	Leu	Val	Ala	Tyr	Ile	Val	Cys	Asp	340	345	350	355
Phe	His	Ser	Leu	Ser	Gly	Phe	Thr	Ser	Ser	Gly	Val	Lys	Val	Ser	360	365	370	375
Ile	Tyr	Ala	Ser	Pro	Asp	Lys	Arg	Asn	Gln	Thr	His	Tyr	Ala	Leu	380	385	390	395
Gln	Ala	Ser	Leu	Lys	Leu	Leu	Asp	Phe	Tyr	Glu	Lys	Tyr	Phe	Asp	400	405	410	415
Ile	Tyr	Tyr	Pro	Leu	Ser	Lys	Leu	Asp	Leu	Ile	Ala	Ile	Pro	Asp	420	425	430	435
Phe	Ala	Pro	Gly	Ala	Met	Glu	Asn	Trp	Gly	Leu	Ile	Thr	Tyr	Arg				
Glu	Thr	Ser	Leu	Leu	Phe	Asp	Pro	Lys	Thr	Ser	Ser	Ala	Ser	Asp				
Lys	Leu	Trp	Val	Thr	Arg	Val	Ile	Ala	His	Glu	Leu	Ala	His	Gln				
Trp	Phe	Gly	Asn	Leu	Val	Thr	Met	Glu	Trp	Trp	Asn	Asp	Ile	Trp				
Leu	Lys	Glu	Gly	Phe	Ala	Lys	Tyr	Met	Glu	Leu	Ile	Ala	Val	Asn				
Ala	Thr	Tyr	Pro	Glu	Leu	Gln	Phe	Asp	Asp	Tyr	Phe	Leu	Asn	Val				
Cys	Phe	Glu	Val	Ile	Thr	Lys	Asp	Ser	Leu	Asn	Ser	Ser	Arg	Pro				

Ile	Ser	Lys	Pro	Ala	Glu	Thr	Pro	Thr	Gln	Ile	Gln	Glu	Met	Phe
				440					445					450
Asp	Glu	Val	Ser	Tyr	Asn	Lys	Gly	Ala	Cys	Ile	Leu	Asn	Met	Leu
				455					460					465
Lys	Asp	Phe	Leu	Gly	Glu	Glu	Lys	Phe	Gln	Lys	Gly	Ile	Ile	Gln
				470					475					480
Tyr	Leu	Lys	Lys	Phe	Ser	Tyr	Arg	Asn	Ala	Lys	Asn	Asp	Asp	Leu
				485					490					495
Trp	Ser	Ser	Leu	Ser	Asn	Ser	Cys	Leu	Glu	Ser	Asp	Phe	Thr	Ser
				500					505					510
Gly	Gly	Val	Cys	His	Ser	Asp	Pro	Lys	Met	Thr	Ser	Asn	Met	Leu
				515					520					525
Ala	Phe	Leu	Gly	Glu	Asn	Ala	Glu	Val	Lys	Glu	Met	Met	Thr	Thr
				530					535					540
Trp	Thr	Leu	Gln	Lys	Gly	Ile	Pro	Leu	Leu	Val	Val	Lys	Gln	Asp
				545					550					555
Gly	Cys	Ser	Leu	Arg	Leu	Gln	Gln	Glu	Arg	Phe	Leu	Gln	Gly	Val
				560					565					570
Phe	Gln	Glu	Asp	Pro	Glu	Trp	Arg	Ala	Leu	Gln	Glu	Arg	Tyr	Leu
				575					580					585
Trp	His	Ile	Pro	Leu	Thr	Tyr	Ser	Thr	Ser	Ser	Ser	Asn	Val	Ile
				590					595					600
His	Arg	His	Ile	Leu	Lys	Ser	Lys	Thr	Asp	Thr	Leu	Asp	Leu	Pro
				605					610					615
Glu	Lys	Thr	Ser	Trp	Val	Lys	Phe	Asn	Val	Asp	Ser	Asn	Gly	Tyr
				620					625					630
Tyr	Ile	Val	His	Tyr	Glu	Gly	His	Gly	Trp	Asp	Gln	Leu	Ile	Thr
				635					640					645
Gln	Leu	Asn	Gln	Asn	His	Thr	Leu	Leu	Arg	Pro	Lys	Asp	Arg	Val
				650					655					660
Gly	Leu	Ile	His	Asp	Val	Phe	Gln	Leu	Val	Gly	Ala	Gly	Arg	Leu
				665					670					675
Thr	Leu	Asp	Lys	Ala	Leu	Asp	Met	Thr	Tyr	Tyr	Leu	Gln	His	Glu
				680					685					690
Thr	Ser	Ser	Pro	Ala	Leu	Leu	Glu	Gly	Leu	Ser	Tyr	Leu	Glu	Ser
				695					700					705
Phe	Tyr	His	Met	Met	Asp	Arg	Arg	Asn	Ile	Ser	Asp	Ile	Ser	Glu
				710					715					720
Asn	Leu	Lys	Arg	Tyr	Leu	Leu	Gln	Tyr	Phe	Lys	Pro	Val	Ile	Asp
				725					730					735
Arg	Gln	Ser	Trp	Ser	Asp	Lys	Gly	Ser	Val	Trp	Asp	Arg	Met	Leu
				740					745					750
Arg	Ser	Ala	Leu	Leu	Lys	Leu	Ala	Cys	Asp	Leu	Asn	His	Ala	Pro
				755					760					765
Cys	Ile	Gln	Lys	Ala	Ala	Glu	Leu	Phe	Ser	Gln	Trp	Met	Glu	Ser
				770					775					780
Ser	Gly	Lys	Leu	Asn	Ile	Pro	Thr	Asp	Val	Leu	Lys	Ile	Val	Tyr
				785					790					795
Ser	Val	Gly	Ala	Gln	Thr	Thr	Ala	Gly	Trp	Asn	Tyr	Leu	Leu	Glu
				800					805					810
Gln	Tyr	Glu	Leu	Ser	Met	Ser	Ser	Ala	Glu	Gln	Asn	Lys	Ile	Leu
				815					820					825
Tyr	Ala	Leu	Ser	Thr	Ser	Lys	His	Gln	Glu	Lys	Leu	Leu	Lys	Leu
				830					835					840
Ile	Glu	Leu	Gly	Met	Glu	Gly	Lys	Val	Ile	Lys	Thr	Gln	Asn	Leu
				845					850					855
Ala	Ala	Leu	Leu	His	Ala	Ile	Ala	Arg	Arg	Pro	Lys	Gly	Gln	Gln
				860					865					870
Leu	Ala	Trp	Asp	Phe	Val	Arg	Glu	Asn	Trp	Thr	His	Leu	Leu	Lys
				875					880					885
Lys	Phe	Asp	Leu	Gly	Ser	Tyr	Asp	Ile	Arg	Met	Ile	Ile	Ser	Gly
				890					895					900
Thr	Thr	Ala	His	Phe	Ser	Ser	Lys	Asp	Lys	Leu	Gln	Glu	Val	Lys
				905					910					915
Leu	Phe	Phe	Glu	Ser	Leu	Glu	Ala	Gln	Gly	Ser	His	Leu	Asp	Ile
				920					925					930
Phe	Gln	Thr	Val	Leu	Glu	Thr	Ile	Thr	Lys	Asn	Ile	Lys	Trp	Leu

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Glu Lys Asn Leu	Pro Thr Leu Arg Thr	Trp Leu Met Val Asn	Thr		
	950	955		960	

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 Ser Tyr Phe Ser Gln Phe Arg Glu Glu Arg Gly Gly Gly Phe Leu  
 35 40 45  
 Cys Phe His Tyr Arg His Arg Pro Glu Arg Ala Pro Pro Gln Ala  
 50 55 60  
 Ala Pro Asn Ser Ala Leu Ile Pro Thr Asp Pro Ala Ala Glu Gly  
 65 70 75  
 Gln Leu Leu Ser Gln Thr Ser Ala Thr Asp Val Arg Pro Leu Ser  
 80 85 90  
 Thr Arg Asp Ser Thr Pro Ile Gln Thr Arg Thr Cys Cys Cys Val  
 95 100 105  
 Ile Ser Val Arg Gly Leu Ala Gln Ala Gln Arg Leu Ile Arg Met  
 110 115 120  
 Tyr Ser Gly Arg Arg Trp Leu Asp Ser His Gly Thr Trp Leu Pro  
 125 130 135  
 Gly Arg Cys Leu Ile Arg Arg Leu Arg Leu Pro Thr Glu Ala Ser  
 140 145 150  
 Gly Leu Gly Ser Phe Pro Phe Lys Thr Arg Lys Glu Leu Gln Ser  
 155 160 165  
 Trp Lys Ala Glu Asn Glu Ala Phe Thr Leu Ala Asp Leu Lys Gln  
 170 175 180  
 Leu Pro Glu Leu Asn Pro Pro Val Leu Met Pro Arg Gly Asn Val  
 185 190 195  
 Gly Thr Pro Leu Arg Val Phe Leu Glu Leu Ile Arg Ala Cys Arg  
 200 205 210  
 Leu Pro Pro Arg Ile Ile Thr Gln Leu Gln Leu Gln Phe Pro Lys  
 215 220 225  
 Thr Gly Ser Ser Arg Arg Tyr Gly Asn Val Pro Phe Glu Tyr Glu  
 230 235 240  
 Asp Ser Glu Thr Val Glu Gln Glu Glu Leu Val Tyr Thr Ala Glu  
 245 250 255  
 Gly Glu Glu Ile Pro Gln Gly Thr Tyr Leu Ala Asp Ile Pro Ala  
 260 265 270  
 Ser Pro Cys Gly Glu Pro Glu Glu Glu Val Gly Lys Glu Glu Glu  
 275 280 285  
 Glu Glu Ser His Ser Asp Glu Asp Asp Asp Arg Gly Glu Glu Trp  
 290 295 300  
 Glu Arg His Glu Ala Leu His Glu Asp Val Thr Gly Gln Glu Arg  
 305 310 315  
 Thr Thr Glu Gln Leu Phe Glu Glu Glu Ile Glu Leu Lys Trp Glu  
 320 325 330  
 Lys Gly Gly Ser Gly Leu Val Phe Tyr Thr Asp Ala Gln Phe Trp  
 335 340 345  
 Gln Glu Glu Glu Gly Asp Phe Asp Glu Gln Thr Ala Asp Asp Trp  
 350 355 360  
 Asp Val Asp Met Ser Val Tyr Tyr Asp Arg Asp Gly Gly Asp Lys  
 365 370 375  
 Asp Ala Arg Asp Ser Val Gln Met Arg Leu Glu Gln Arg Leu Arg  
 380 385 390

Asp	Gly	Gln	Glu	Asp	Gly	Ser	Val	Ile	Glu	Arg	Gln	Val	Gly	Thr
				395					400					405
Phe	Glu	Arg	His	Thr	Lys	Gly	Ile	Gly	Arg	Lys	Val	Met	Glu	Arg
				410					415					420
Gln	Gly	Trp	Ala	Glu	Gly	Gln	Gly	Leu	Gly	Cys	Arg	Cys	Ser	Gly
				425					430					435
Val	Pro	Glu	Ala	Leu	Asp	Ser	Asp	Gly	Gln	His	Pro	Arg	Cys	Lys
				440					445					450
Arg	Gly	Leu	Gly	Tyr	His	Gly	Glu	Lys	Leu	Gln	Pro	Phe	Gly	Gln
				455					460					465
Leu	Lys	Arg	Pro	Arg	Arg	Asn	Gly	Leu	Gly	Leu	Ile	Ser	Thr	Ile
				470					475					480
Tyr	Asp	Glu	Pro	Leu	Pro	Gln	Asp	Gln	Thr	Glu	Ser	Leu	Leu	Arg
				485					490					495
Arg	Gln	Pro	Pro	Thr	Ser	Met	Lys	Phe	Arg	Thr	Asp	Met	Ala	Phe
				500					505					510
Val	Arg	Gly	Ser	Ser	Cys	Ala	Ser	Asp	Ser	Pro	Ser	Leu	Pro	Asp
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&lt;211&gt; 795

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

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&lt;223&gt; Incyte ID No: 1872095CD1

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Lys	Asp	Ala	Ser	Met	Thr	Gln	Ala	Leu	Cys	Arg	Met	Ile	Asp	Trp
				20					25					30
Leu	Ser	Trp	Pro	Leu	Ala	Gln	His	Val	Asp	Thr	Trp	Val	Ile	Ala
				35					40					45
Leu	Leu	Lys	Gly	Leu	Ala	Ala	Val	Gln	Lys	Phe	Thr	Ile	Leu	Ile
				50					55					60
Asp	Val	Thr	Leu	Leu	Lys	Ile	Glu	Leu	Val	Phe	Asn	Arg	Leu	Trp
				65					70					75
Phe	Pro	Leu	Val	Arg	Pro	Gly	Ala	Leu	Ala	Val	Leu	Ser	His	Met
				80					85					90
Leu	Leu	Ser	Phe	Gln	His	Ser	Pro	Glu	Ala	Phe	His	Leu	Ile	Val
				95					100					105
Pro	His	Val	Val	Asn	Leu	Val	His	Ser	Phe	Lys	Asn	Asp	Gly	Leu
				110					115					120
Pro	Ser	Ser	Thr	Ala	Phe	Leu	Val	Gln	Leu	Thr	Glu	Leu	Ile	His
				125					130					135
Cys	Met	Met	Tyr	His	Tyr	Ser	Gly	Phe	Pro	Asp	Leu	Tyr	Glu	Pro
				140					145					150
Ile	Leu	Glu	Ala	Ile	Lys	Asp	Phe	Pro	Lys	Pro	Ser	Glu	Glu	Lys
				155					160					165
Ile	Lys	Leu	Ile	Leu	Asn	Gln	Ser	Ala	Trp	Thr	Ser	Gln	Ser	Asn
				170					175					180
Ser	Leu	Ala	Ser	Cys	Leu	Ser	Arg	Leu	Ser	Gly	Lys	Ser	Glu	Thr
				185					190					195
Gly	Lys	Thr	Gly	Leu	Ile	Asn	Leu	Gly	Asn	Thr	Cys	Tyr	Met	Asn
				200					205					210
Ser	Val	Ile	Gln	Ala	Leu	Phe	Met	Ala	Thr	Asp	Phe	Arg	Arg	Gln
				215					220					225
Val	Leu	Ser	Leu	Asn	Leu	Asn	Gly	Cys	Asn	Ser	Leu	Met	Lys	Lys
				230					235					240
Leu	Gln	His	Leu	Phe	Ala	Phe	Leu	Ala	His	Thr	Gln	Arg	Glu	Ala
				245					250					255
Tyr	Ala	Pro	Arg	Ile	Phe	Phe	Glu	Ala	Ser	Arg	Pro	Pro	Trp	Phe
				260					265					270
Thr	Pro	Arg	Ser	Gln	Gln	Asp	Cys	Ser	Glu	Tyr	Leu	Arg	Phe	Leu

Leu Asp Arg Leu	275	Glu Glu Glu Lys	280	Ile Leu Lys Val Gln	285
Ser His Lys Pro	290	Ser Glu Ile Leu Glu	295	Cys Ser Glu Thr Ser	300
Gln Glu Val Ala	305	Ser Lys Ala Ala Val	310	Leu Thr Glu Thr Pro	315
Thr Ser Asp Gly	320	Glu Lys Thr Leu Ile	325	Glu Lys Met Phe Gly	330
Lys Leu Arg Thr	335	His Ile Arg Cys Leu	340	Asn Cys Arg Ser Thr	345
Gln Lys Val Glu	350	Ala Phe Thr Asp Leu	355	Ser Leu Ala Phe Cys	360
Ser Ser Ser Leu	365	Glu Asn Met Ser Val	370	Gln Asp Pro Ala Ser	375
Pro Ser Ile Gln	380	Asp Gly Gly Leu Met	385	Gln Ala Ser Val Pro	390
Pro Ser Glu Glu	395	Pro Val Val Tyr Asn	400	Pro Thr Thr Ala Ala	405
Ile Cys Asp Ser	410	Leu Val Asn Glu Lys	415	Thr Ile Gly Ser Pro	420
Asn Glu Phe Tyr	425	Cys Ser Glu Asn Thr	430	Ser Val Pro Asn Glu	435
Asn Lys Ile Leu	440	Val Asn Lys Asp Val	445	Pro Gln Lys Pro Gly	450
Glu Thr Thr Pro	455	Ser Val Thr Asp Leu	460	Leu Asn Tyr Phe Leu	465
Pro Glu Ile Leu	470	Thr Gly Asp Asn Gln	475	Tyr Tyr Cys Glu Asn	480
Ala Ser Leu Gln	485	Asn Ala Glu Lys Thr	490	Met Gln Ile Thr Glu	495
Pro Glu Tyr Leu	500	Ile Leu Thr Leu Leu	505	Arg Phe Ser Tyr Asp	510
Lys Tyr His Val	515	Arg Arg Lys Ile Leu	520	Asp Asn Val Ser Leu	525
Leu Val Leu Glu	530	Leu Pro Val Lys Arg	535	Ile Thr Ser Phe Ser	540
Leu Ser Glu Ser	545	Trp Ser Val Asp Val	550	Asp Phe Thr Asp Leu	555
Glu Asn Leu Ala	560	Lys Lys Leu Lys Pro	565	Ser Gly Thr Asp Glu	570
Ser Cys Thr Lys	575	Leu Val Pro Tyr Leu	580	Leu Ser Ser Val Val	585
His Ser Gly Ile	590	Ser Ser Glu Ser Gly	595	His Tyr Tyr Ser Tyr	600
Arg Asn Ile Thr	605	Ser Thr Asp Ser Ser	610	Tyr Gln Met Tyr His	615
Ser Glu Ala Leu	620	Ala Leu Ala Ser Ser	625	Gln Ser His Leu Leu	630
Arg Asp Ser Pro	635	Ser Ala Val Phe Glu	640	Gln Asp Leu Glu Asn	645
Glu Met Ser Lys	650	Glu Trp Phe Leu Phe	655	Asn Asp Ser Arg Val	660
Phe Thr Ser Phe	665	Gln Ser Val Gln Lys	670	Ile Thr Ser Arg Phe	675
Lys Asp Thr Ala	680	Tyr Val Leu Leu Tyr	685	Lys Lys Gln His Ser	690
Asn Gly Leu Ser	695	Gly Asn Asn Pro Thr	700	Ser Gly Leu Trp Ile	705
Gly Asp Pro Pro	710	Leu Gln Lys Glu Leu	715	Met Asp Ala Ile Thr	720
Asp Asn Lys Leu	725	Tyr Leu Gln Glu Gln	730	Glu Leu Asn Ala Arg	735
Arg Ala Leu Gln	740	Ala Ala Ser Ala Ser	745	Cys Ser Phe Arg Pro	750
Gly Phe Asp Asp	755	Asn Asp Pro Pro Gly	760	Ser Cys Gly Pro Thr	765
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Gly Gly Gly Gly Gly Gly Phe Asn Thr Val Gly Arg Leu Val Phe  
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 Arg Ser Ser Arg Arg Arg Leu His Ser Leu Gly Leu Ala Ala Met  
 35 40 45  
 Pro Glu Lys Arg Pro Phe Glu Arg Leu Pro Ala Asp Val Ser Pro  
 50 55 60  
 Ile Asn Cys Ser Leu Cys Leu Lys Pro Asp Leu Leu Asp Phe Thr  
 65 70 75  
 Phe Glu Gly Lys Leu Glu Ala Ala Ala Gln Val Arg Gln Ala Thr  
 80 85 90  
 Asn Gln Ile Val Met Asn Cys Ala Asp Ile Asp Ile Ile Thr Ala  
 95 100 105  
 Ser Tyr Ala Pro Glu Gly Asp Glu Glu Ile His Ala Thr Gly Phe  
 110 115 120  
 Asn Tyr Gln Asn Glu Asp Glu Lys Val Thr Leu Ser Phe Pro Ser  
 125 130 135  
 Thr Leu Gln Thr Gly Thr Gly Thr Leu Lys Ile Asp Phe Val Gly  
 140 145 150  
 Glu Leu Asn Asp Lys Met Lys Gly Phe Tyr Arg Ser Lys Tyr Thr  
 155 160 165  
 Thr Pro Ser Gly Glu Val Arg Tyr Ala Ala Val Thr Gln Phe Glu  
 170 175 180  
 Ala Thr Asp Ala Arg Arg Ala Phe Pro Cys Trp Asp Glu Pro Ala  
 185 190 195  
 Ile Lys Ala Thr Phe Asp Ile Ser Leu Val Val Pro Lys Asp Arg  
 200 205 210  
 Val Ala Leu Ser Asn Met Asn Val Ile Asp Arg Lys Pro Tyr Pro  
 215 220 225  
 Asp Asp Glu Asn Leu Val Glu Val Lys Phe Ala Arg Thr Pro Val  
 230 235 240  
 Met Ser Thr Tyr Leu Val Ala Phe Val Val Gly Glu Tyr Asp Phe  
 245 250 255  
 Val Glu Thr Arg Ser Lys Asp Gly Val Cys Val Arg Val Tyr Thr  
 260 265 270  
 Pro Val Gly Lys Ala Glu Gln Gly Lys Phe Ala Leu Glu Val Ala  
 275 280 285  
 Ala Lys Thr Leu Pro Phe Tyr Lys Asp Tyr Phe Asn Val Pro Tyr  
 290 295 300  
 Pro Leu Pro Lys Ile Asp Leu Ile Ala Ile Ala Asp Phe Ala Ala  
 305 310 315  
 Gly Ala Met Glu Asn Trp Gly Leu Val Thr Tyr Arg Glu Thr Ala  
 320 325 330  
 Leu Leu Ile Asp Pro Lys Asn Ser Cys Ser Ser Arg Gln Trp  
 335 340 345  
 Val Ala Leu Val Val Gly His Glu Leu Ala His Gln Trp Phe Gly  
 350 355 360  
 Asn Leu Val Thr Met Glu Trp Trp Thr His Leu Trp Leu Asn Glu  
 365 370 375  
 Gly Phe Ala Ser Trp Ile Glu Tyr Leu Cys Val Asp His Cys Phe  
 380 385 390  
 Pro Glu Tyr Asp Ile Trp Thr Gln Phe Val Ser Ala Asp Tyr Thr



Arg Ala Gln Glu	395	Leu Asp Ala Leu Asp	400	Asn Ser His Pro Ile	405
Val Ser Val Gly	410	His Pro Ser Glu Val	415	Glu Ile Phe Asp	420
Ile Ser Tyr Ser	425	Lys Gly Ala Ser Val	430	Ile Arg Met Leu His	435
Tyr Ile Gly Asp	440	Lys Asp Phe Lys Lys	445	Gly Met Asn Met Tyr	450
Thr Lys Phe Gln	455	Gln Lys Asn Ala Ala	460	Thr Glu Asp Leu Trp	465
Ser Leu Glu Asn	470	Ala Ser Gly Lys Pro	475	Ile Ala Ala Val Met	480
Thr Trp Thr Lys	485	Met Gly Phe Pro	490	Ile Tyr Val Glu	495
Glu Gln Val Glu	500	Asp Asp Arg Leu Leu	505	Leu Ser Gln Lys	510
Phe Cys Ala Gly	515	Gly Ser Tyr Val Gly	520	Glu Asp Cys Pro Gln	525
Met Val Pro Ile	530	Thr Ile Ser Thr Ser	535	Glu Asp Pro Asn Gln	540
Lys Leu Lys Ile	545	Leu Met Asp Lys Pro	550	Glu Met Asn Val Val	555
Lys Asn Val Lys	560	Pro Asp Gln Trp Val	565	Lys Leu Asn Leu Gly	570
Val Gly Phe Tyr	575	Arg Thr Gln Tyr Ser	580	Ser Ala Met Leu Glu	585
Leu Leu Pro Gly	590	Ile Arg Asp Leu Ser	595	Leu Pro Pro Val Asp	600
Leu Gly Leu Gln	605	Asn Asp Leu Phe Ser	610	Leu Ala Arg Ala Gly	615
Ile Ser Thr Val	620	Glu Val Leu Lys Val	625	Met Glu Ala Phe Val	630
Glu Pro Asn Tyr	635	Thr Val Trp Ser Asp	640	Leu Ser Cys Asn Leu	645
Ile Leu Ser Thr	650	Leu Leu Ser His Thr	655	Asp Phe Tyr Glu Glu	660
Gln Glu Phe Val	665	Lys Asp Val Phe Ser	670	Pro Ile Gly Glu Arg	675
Gly Trp Asp Pro	680	Lys Pro Gly Glu Gly	685	His Leu Asp Ala Leu	690
Arg Gly Leu Val	695	Leu Gly Lys Leu Gly	700	Lys Ala Gly His Lys	705
Thr Leu Glu Glu	710	Ala Arg Arg Arg Phe	715	Lys Asp His Val Glu	720
Lys Gln Ile Leu	725	Ser Ala Asp Leu Arg	730	Ser Pro Val Tyr Leu	735
Val Leu Lys His	740	Gly Asp Gly Thr Thr	745	Leu Asp Ile Met Leu	750
Leu His Lys Gln	755	Ala Asp Met Gln Glu	760	Glu Lys Asn Arg Ile	765
Arg Val Leu Gly	770	Ala Thr Leu Leu Pro	775	Asp Leu Ile Gln Lys	780
Leu Thr Phe Ala	785	Leu Ser Glu Glu Val	790	Arg Pro Gln Asp Thr	795
Ser Val Ile Gly	800	Gly Val Ala Gly Gly	805	Ser Lys His Gly Arg	810
Ala Ala Trp Lys	815	Phe Ile Lys Asp Asn	820	Trp Glu Glu Leu Tyr	825
Arg Tyr Gln Gly	830	Gly Phe Leu Ile Ser	835	Arg Leu Ile Lys Leu	840
Val Glu Gly Phe	845	Ala Val Asp Lys Met	850	Ala Gly Glu Val Lys	855
Phe Phe Glu Ser	860	His Pro Ala Pro Ser	865	Ala Glu Arg Thr Ile	870
Gln Cys Cys Glu	875	Asn Ile Leu Leu Asn	880	Ala Ala Trp Leu Lys	885
	890		895		900

Asp Ala Glu Ser Ile His Gln Tyr Leu Leu Gln Arg Lys Ala Ser  
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 Pro Pro Thr Val

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 35 40 45  
 Glu Ile Pro Val Glu Tyr Gln Thr Val Gln Arg Leu Ile Pro Ala  
 50 55 60  
 Leu Trp Glu Lys His Ser Pro Gln Leu Val Val His Val Gly Val  
 65 70 75  
 Ser Gly Met Ala Thr Thr Val Thr Leu Glu Lys Cys Gly His Asn  
 80 85 90  
 Lys Gly Tyr Lys Gly Leu Asp Asn Cys Arg Phe Cys Pro Gly Ser  
 95 100 105  
 Gln Cys Cys Val Glu Asp Gly Pro Glu Ser Ile Asp Ser Ile Ile  
 110 115 120  
 Asp Met Asp Ala Val Cys Lys Arg Val Thr Thr Leu Gly Leu Asp  
 125 130 135  
 Val Ser Val Thr Ile Ser Gln Asp Ala Gly Arg Lys Lys Pro Phe  
 140 145 150  
 Pro Ala Lys Gly Asp Cys Val Phe Cys Arg Arg Arg Arg Ala Arg  
 155 160 165  
 Ser Leu Gln Ala Gln Cys Gly Phe Ser Leu Thr Pro Ala Leu Glu  
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 <213> Homo sapiens

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 <223> Incyte ID No: 3937958CD1

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 Cys Trp Pro Arg Tyr Asn Lys Asp Glu Lys Tyr Leu Gln Leu Asp  
 35 40 45  
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 Gln Phe

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 <223> Incyte ID No: 7257324CD1

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 35 40 45  
 Lys Glu Leu Ala Arg Gln Asn Met Asp Leu Gly Phe Lys Leu Leu  
 50 55 60  
 Lys Lys Leu Ala Phe Tyr Asn Pro Gly Arg Asn Ile Phe Leu Ser  
 65 70 75  
 Pro Leu Ser Ile Ser Thr Ala Phe Ser Met Leu Cys Leu Gly Ala  
 80 85 90  
 Gln Asp Ser Thr Leu Asp Glu Ile Lys Gln Gly Phe Asn Phe Arg  
 95 100 105  
 Lys Met Pro Glu Lys Asp Leu His Glu Gly Phe His Tyr Ile Ile  
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 His Glu Leu Thr Gln Lys Thr Gln Asp Leu Lys Leu Ser Ile Gly  
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 Asn Thr Leu Phe Ile Asp Gln Arg Leu Gln Pro Gln Arg Lys Phe  
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 Asn Phe Gln Asn Leu Glu Met Ala Gln Lys Gln Ile Asn Asp Phe  
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 Ile Ser Gln Lys Thr His Gly Lys Ile Asn Asn Leu Ile Glu Asn  
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 Ile Asp Pro Gly Thr Val Met Leu Leu Ala Asn Tyr Ile Phe Phe  
 200 205 210  
 Arg Ala Arg Trp Lys His Glu Phe Asp Pro Asn Val Thr Lys Glu  
 215 220 225  
 Glu Asp Phe Phe Leu Glu Lys Asn Ser Ser Val Lys Val Pro Met  
 230 235 240  
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 245 250 255  
 Ser Cys Thr Ile Leu Glu Ile Pro Tyr Gln Lys Asn Ile Thr Ala  
 260 265 270  
 Ile Phe Ile Leu Pro Asp Glu Gly Lys Leu Lys His Leu Glu Lys  
 275 280 285  
 Gly Leu Gln Val Asp Thr Phe Ser Arg Trp Lys Thr Leu Leu Ser  
 290 295 300  
 Arg Arg Val Val Asp Val Ser Val Pro Arg Leu His Met Thr Gly  
 305 310 315  
 Thr Phe Asp Leu Lys Lys Thr Leu Ser Tyr Ile Gly Val Ser Lys  
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 Ser Leu Lys Val Gly Glu Ala Val His Lys Ala Glu Leu Lys Met  
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 Asp Glu Arg Gly Thr Glu Gly Ala Ala Gly Thr Gly Ala Gln Thr  
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 Leu Pro Met Glu Thr Pro Leu Val Val Lys Ile Asp Lys Pro Tyr  
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 Trp Asn Ser Ala Val Lys Ser Thr Pro Leu Ser Asn Tyr Leu Asp  
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 Ala Gln Tyr Phe Gly Pro Ile Thr Ile Gly Thr Pro Pro Gln Thr  
 80 85 90  
 Phe Lys Val Ile Phe Asp Thr Gly Ser Ser Asn Leu Trp Val Pro  
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 Ser Ala Thr Cys Ala Ser Thr Met Val Ala Cys Arg Val His Asn  
 110 115 120  
 Arg Tyr Phe Ala Lys Arg Ser Thr Ser His Gln Val Arg Gly Asp  
 125 130 135  
 His Phe Ala Ile His Tyr Gly Ser Gly Ser Leu Ser Gly Phe Leu  
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 Ser Thr Asp Thr Val Arg Val Ala Gly Leu Glu Ile Arg Asp Gln  
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 Thr Phe Ala Glu Ala Thr Glu Met Pro Gly Pro Ile Phe Leu Ala  
 170 175 180  
 Ala Lys Phe Asp Gly Ile Phe Gly Leu Ala Tyr Arg Ser Ile Ser  
 185 190 195  
 Met Gln Arg Ile Lys Pro Pro Phe Tyr Ala Met Met Glu Gln Gly  
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 215 220 225  
 Glu Lys Asp Gly Gly Ala Ile Phe Phe Gly Gly Ser Asn Pro His  
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 Tyr Tyr Thr Gly Asn Phe Thr Tyr Val Gln Val Ser His Arg Ala  
 245 250 255  
 Tyr Trp Gln Val Lys Met Asp Ser Ala Val Ile Arg Asn Leu Glu  
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 275 280 285  
 Phe Leu Ala Leu Pro Tyr Asp Gln Ala Ile Leu Ile Asn Glu Ser  
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 305 310 315  
 Asp Ser Val Pro Asp Leu Pro Lys Ile Thr Phe Thr Leu Gly Gly  
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 335 340 345  
 Tyr Gln Asp Arg Arg Ile Cys Ser Ser Ala Phe Ile Ala Val Asp  
 350 355 360  
 Leu Pro Ser Pro Ser Gly Pro Leu Trp Ile Leu Gly Asp Val Phe  
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&lt;221&gt; misc\_feature

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Phe	Gln	Gln	Lys	Leu	Met	Ser	Lys	Lys	Asn	Met	Asn	Ser	Thr	Leu
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Cys	Lys	Arg	Asn	Asp	Thr	Ser	Asn	Ser	Ser	Cys	Pro	Leu	Gln	Ser
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Lys	Lys	Leu	Arg	Lys	Ser	Leu	Ile	Cys	Glu	Ser	Leu	Ile	Tyr	Thr
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&lt;211&gt; 4028

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 1714846CB1

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&lt;210&gt; 17

&lt;211&gt; 1911

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2617672CB1

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&lt;211&gt; 854

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 2769104CB1

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:  
11 July 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **PROTEASES**

(57) Abstract: The invention provides human proteases (PRTS) and polynucleotides which identify and encode PRTS. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of PRTS.

WO 01/046443 A3

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/34811

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/57 C12N9/48 A01K67/00 C07K16/40 C12Q1/68  
 A61K38/48 G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01K C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, EMBL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VALERO REBECA ET AL: "USP25, a novel gene encoding a deubiquitinating enzyme, is located in the gene-poor region 21q11.2" GENOMICS, ACADEMIC PRESS, SAN DIEGO, US, vol. 62, no. 3, 15 December 1999 (1999-12-15), pages 395-405, XP002153277            ISSN: 0888-7543            the whole document</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-7, 11-15

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

4 September 2001

Date of mailing of the international search report

09 01 2002

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ESPEN, J



## INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/US 00/34811

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	GROET J ET AL: "NARROWING OF THE REGIONS OF ALLELIC LOSS IN 21Q11-21 IN SQUAMOUS NON-SMALL CELL LUNG CARCINOMA AND CLONING OF A NOVEL UBIQUITIN-SPECIFIC PROTEASE GENE FROM THE DELETED SEGMENT" GENES, CHROMOSOMES & CANCER, XX, XX, vol. 27, no. 2, February 2000 (2000-02), pages 153-161, XP000943091 the whole document	1-7, 11-15
X	WO 99 46289 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); ROSEN CRAIG A (US); FERRI) 16 September 1999 (1999-09-16) SEQ ID NO 130 claims 1-23	1-7, 9-18,25, 26
E	WO 00 78934 A (GROET JUERGEN ;NIZETIC DEAN (GB); UNIV LONDON PHARMACY (GB)) 28 December 2000 (2000-12-28) SEQ ID NOs 1,6 claims 1-35	1-18
E	WO 00 79267 A (GROET JUERGEN ;NIZETIC DEAN (GB); UNIV LONDON PHARMACY (GB)) 28 December 2000 (2000-12-28) SEQ ID NO 1,6 claims 1-11	1-7,9, 11-18
E	WO 01 21654 A (RIGEL PHARMACEUTICALS INC ;LUO YING (US); MANCEBO HELENA (US)) 29 March 2001 (2001-03-29) SEQ ID NO 1 claims 1-22; figure 2	1-7, 9-14, 25-27
X	DATABASE EMBL [Online] Database entry HS1271038; Accession no AA479313, 23 June 1997 (1997-06-23) HILLIER L ET AL: "Homo sapiens cDNA clone" XP002176711 sequence	11,12
X	DATABASE EMBL [Online] Database entry HS1272487, Accession no AA482201, 24 June 1997 (1997-06-24) NATIONAL CANCER INSTITUTE, CANCER GEMONE ANATOMY PROJECT (CGAP): "Homo sapiens cDNA clone similar to ubiquitin carboxyl-terminal hydrolase 2" XP002176712 sequence	11,12

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/34811

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL [Online] Database entry HSZZ12939, Accession no AA307805, 18 April 1997 (1997-04-18) ADAMS MD ET AL: "EST178661 Colon carcinoma (HCC) cell line Homo sapiens cDNA" XP002176713 sequence</p> <p>-----</p>	11,12

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/34811

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☒ Claims Nos.: 20,21,23,24  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

In part 1-28

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20,21,23,24

Claim 20 refers to an agonist of a polypeptide of claim 1 and claim 23 refers to an antagonist of a polypeptide of claim 1 without giving a true technical characterization. Moreover, no such specific compounds are defined in the application. In consequence, the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).

No search can be carried out for such purely speculative claims whose wording is in fact, a mere recitation of the result to be achieved. The above comments also applies for claims 21 and 24.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.